15 JUN 2000 **526 Rec'd PCT/PTO** U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE FORM PTO 1390 (Modified) REV 11-98) 002.00120 TRANSMITTAL LETTER TO THE UNITED STATES U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR DESIGNATED/ELECTED OFFICE (DO/EO/US) 581651 CONCERNING A FILING UNDER 35 U.S.C. 371 PRIORITY DATE CLAIMED INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE 16 December 1997 PCT/GB98/03766 **15 December 1998** TITLE OF INVENTION POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF APPLICANT(S) FOR DO/EO/US Schor, Seth Lawrence Schor, Ana Maria Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).  $\times$ 3. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 4. A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) is transmitted herewith (required only if not transmitted by the International Bureau). a. 🗆 b. 🗵 has been transmitted by the International Bureau. is not required, as the application was filed in the United States Receiving Office (RO/US). c. 🗆 A translation of the International Application into English (35 U.S.C. 371(c)(2)).  $\times$ A copy of the International Search Report (PCT/ISA/210). Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) are transmitted herewith (required only 1f not transmitted by the International Bureau). a. 🗆 have been transmitted by the International Bureau. have not been made; however, the time limit for making such amendments has NOT expired.  $\boxtimes$ have not been made and will not be made. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 10. An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). A copy of the International Preliminary Examination Report (PCT/IPEA/409). A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 12. (35 U.S.C. 371 (c)(5)). Items 13 to 20 below concern document(s) or information included: An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 13. П An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 14. 15. A FIRST preliminary amendment. A SECOND or SUBSEQUENT preliminary amendment. 16. 17. A substitute specification.

- 18. 

  A change of power of attorney and/or address letter.
- 20. 

  Other items or information:

Ē

Communication (1 pg)

527 Rec'd PCT/PTO 15 JUN 2000

U.S. APPLICATION NO JIF INDEWS SEE 7 SR INTERNATIONAL AI						DOCKET NUMBER .00120
21. The following fees are submitted:.					CALCULATION	S PTO USE ONLY
BASIC NATIONA  Neither inter	L FEE (37 CFR 1.492 (a) (1) - mational preliminary examination search fee (37 CFR 1.445(a)(2))	n fee (37 CFR 1.482) nor paid to USPTO	005	vo 00		110 000 0101
and International Search Report not prepared by the EPO or JPO						
USPTO but Internation Search Report prepared by the EPO or JPO						
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO						
but all claims did not satisfy provisions of PCT Article 33(1)-(4)						
and all claim	ns satisfied provisions of PCT Art	icle 33(1)-(4)		6.00	6040.00	:
Surcharge of \$130.0	00 for furnishing the oath or decla	aration later than	20 🗵 30		\$840.00	
months from the ear	liest claimed priority date (37 Cl	FR 1.492 (e)).  NUMBER EXTRA	RATE	3	\$130.00	
Total claims	98 - 20 =	78	x \$18.0		\$1,404.00	
Independent claims	24 - 3=	21	x \$78.0	00	\$1,638.00	
Multiple Dependent Claims (check if applicable).					\$260.00	
21/0		ABOVE CALCULA		=	\$4,272.00	
Reduction of 1/2 for must also be filed (	r filing by small entity, if applica Note 37 CFR 1.9, 1.27, 1.28) (ch	eck if applicable).	atement		\$0.00	
		SUI	BTOTAL	=	\$4,272.00	
Processing fee of \$1 months from the ear	<b>30.00</b> for furnishing the English cliest claimed priority date (37 Cl	translation later than . FR 1.492 (f)).	20 🗆 30	0 +	\$0.00	
		TOTAL NATIONA	L FEE	=	\$4,272.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).					\$0.00	
		TOTAL FEES ENC	LOSED		\$4,272.00	
					Amount to be: refunded	\$
					charged	\$
. <del></del> :	the amount of \$4,272.00  rge my Deposit Account No.	to cover the above fees is en			to cover the abov	ve fees.
The Comm	e copy of this sheet is enclosed.			edit an	y overpayment	
•		A duplicate copy of this sheet			n to worder Off CEE	<b>.</b>
	appropriate time limit under 3° ist be filed and granted to restor			petitio	on to revive (37 CFF	(
SEND ALL CORRI	ESPONDENCE TO:		-5	, ,,	9. Bin	
Susan J. Braman Braman & Rogals	skyj, LLP		SIGNAT			
P.O. Box 352 Canandaigus Naw York 14424-0352			Susan J. Braman			
Canandaigua, New York 14424-0352 Tel 716-393-3002			NAME			
Fax 716-393-3001			34,103			
			REGISTI	RATIC	N NUMBER	
			15	9	vne 2000	
			DATE	0		
		ı				

09/581651 527 Rec'd PCT/PTO 15 JUN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Schor and Schor

Serial No.: To be assigned (U.S. National Stage of PCT/GB98/03766)

Filed: Herewith

For: POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

### PRELIMINARY AMENDMENT

Assistant Commissioner for Patents Washington, D.C. 20231 Box PCT

# Dear Sir:

Please amend the subject application as follows (amendments refer to the claims as published; please disregard the amendments made during preliminary examination before the European Patent Office):

# In the Claims:

Please amend claims 3-8, 13, 24, 35, 39, 44, 47, 49, and 51-58 as follows:

- 3. (Amended) A polynucleotide according to Claim 1 [or 2], which contains no introns.
- 4. (Amended) A polynucleotide according to [any one of the preceding claims] <u>Claim 1</u>, comprising the polynucleotide whose sequence is shown in Figure 1.
- 5. (Amended) A polynucleotide according to [any one of the preceding claims] Claim 1, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.
- 6. (Amended) A polynucleotide according to [any one of the preceding claims] Claim 1, encoding a polynucleotide which has migration stimulating factor activity.
- 7. (Amended) A replicable vector comprising a polynucleotide as defined in [any one of Claims 1 to 6] Claim 1.

- 8. (Amended) A host cell comprising a recombinant polynucleotide as defined in Claim 1 or a replicable vector comprising the polynucleotide [as defined in any one of Claims 1 to 7].
- 13. (Amended) A polypeptide according to <u>Claim 10</u> [any one of Claims 10 to 12], which has migration stimulating factor activity.
- 24. (Amended) An antibody according to any one of Claims 14 to [24] <u>17 and 19 to 22</u> which is a monoclonal antibody.
- 35. (Amended) A polynucleotide according to any one of Claims 31 to 33 [34], wherein the polynucleotide which encodes fibronectin or the polynucleotide which encodes the polypeptide as said or a natural variant thereof is a mRNA or a cDNA.
- 39. (Amended) A method according to any one of Claims 36 to 38, wherein the reagent which can distinguish said polypeptide from fibronectin is an antibody according to any one of Claims 14 to 17 [18].
- 44. (Amended) A method according to any one of Claims 36 to 38 and 40 to 42 [43], wherein the cancer is breast cancer.
- 47. (Amended) A method of modulating cell migration the method comprising administering an effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13] to the site where modulation of cell migration is required.
- 49. (Amended) A method according to Claim 47 [or 48], wherein the site is in a mammalian body.
- 51. (Amended) Use of a polypeptide according to any one of Claims 10  $\underline{\text{and }12}$  [to 13], in the manufacture of an agent for modulating cell migration.
- 52. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], for modulating cell migration.
- 53. (Amended) A method of healing a wound the method comprising administering to the locality of the wound an

Serial No. Not Yet Known (US Nat'l of PCT/GB98/03766) Page 3

effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13].

- 54. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], in the manufacture of a medicament for healing wounds.
- 55. (Amended) Use of a polypeptide according to any one of Claims 10 and 12 [to 13], for healing wounds.
- 56. (Amended) A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 and 12 [to 13] and a pharmaceutically acceptable carrier.
- 57. (Amended) A polypeptide according to any one of Claims 10 and 12 [to 13] for use in medicine.
- 58. (Amended) A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 and 12 [to 13].

Please add new claim 59 as follows:

--59. (New) A polypeptide according to Claim 12, which has migration stimulating factor activity.--

### REMARKS

Claims 1-58 are presented for examination in the subject application, as published. By this preliminary amendment, claims 3-8, 13, 24, 35, 39, 44, 47, 49, and 51-58 have been amended to adjust the dependencies of the claims. New claim 59 has been added.

Respectfully submitted,

Dated: 15 June 2000

Susan J. Braman Reg. No. 34,103

Braman & Rogalskyj, LLP P.O. Box 352

Canandaigua, New York 14424-0352

Telephone: (716) 393-3002 Facsimile: (716) 393-3001

10

15

20

25

WO 99/31233 PCT/GB98/03766

# POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF

The present invention relates to polypeptides, polynucleotides and uses thereof and in particular to migration stimulating factor (MSF).

MSF has been described previously in the following papers. Schor et al (1988) J. Cell Sci. 90: 391-399 shows that foetal and cancer patient fibroblasts produce an autocrine migration stimulating factor not made by normal adult cells. Schor et al (1988) J. Cell Sci. 90: 401-407, shows that fibroblasts from cancer patients display a mixture of both foetal and adult phenotypic characteristics. Schor et al (1989) In Vitro 25: 737-746 describes a mechanism of action of the migration stimulating factor (MSF) produced by fetal and cancer patient fibroblasts and its effect on hyaluronic acid synthesis. Grey et al (1989) Proc. Natl. Acad. Sci. (USA) 86: 2438-2442 describes the purification of the migration stimulating factor produced by fetal and cancer patient fibroblasts but no amino acid sequence information is given. It is suggested that MSF has a molecular weight of 70kDa. Schor & Schor (1990) Cancer Investig. 8: 665-667 describes the characterisation of migration stimulating activity (MSF) and gives evidence for its role in cancer pathogenesis. Picardo et al (1991) Lancet 337: 130-133 describes the presence of migration stimulating activity in the serum of breast cancer patients. Ellis et al (1992) J. Cell Sci. 102: 447-456 describes the antagonistic effects of transforming growth factor-\beta1 and MSF on fibroblast migration and hyaluronic acid synthesis and discusses the possible implications for wound healing. Picardo et al (1992) Exp. Mol. Path. 57: 8-21, describes the identification of migration stimulating factor in wound fluid. Irwin et al (1994) J. Cell Sci. 107: 1333-1346, describes the inter- and intra-site heterogeneity in the expression of fetal-like phenotypic characteristics by gingival fibroblasts

10

15

20

25

WO 99/31233 PCT/GB98/03766

and discusses the potential significance for wound healing. Schor et al (1994) Int J Cancer. 59: 25-32 describes the phenotypic heterogeneity in breast fibroblasts and discusses functional anomaly in fibroblasts from histologically normal tissue adjacent to carcinoma. Schor et al (1991) In: Cell Motility Factors (ed. I Goldberg) pp. 127-146, Birkhauser Press, Basel, describes the heterogeneity amongst fibroblasts in the production of migration stimulating factor (MSF) and discusses implications for cancer Schor et al (1993) In: Cell behaviour: Adhesion and pathogenesis. Motility. (ed. G. Evans, C. Wigley and R. Warn) Society for Experimental Biology Symposium No. 47, pp. 234-251, describes the potential structural homology of MSF to the gelatin-binding domain of fibronectin its potential mode of action and possible function in health and disease. A small amount of partial amino acid sequence is given, but this sequence is similar to fibronectin and, in fact, is not present in the MSF which has now been cloned and sequenced in the present work (see below). It is suggested that MSF activity isolated from foetal fibroblast conditioned medium consists of three proteins, one with an apparent molecular weight of 119kDa and a double of 43 and 33kDa, and, indeed, it was suggested that MSF could be a proteotytic degradation product of fibronectin. Schor (1995) In: Epithelial Mesenchymal Interactions in Cancer (eg. I Goldberg and E Rosen). pp. 273-296. Birkhauser Press, Basel, describes fibroblast subpopulations as accelerators of tumor progression and the potential role of migration stimulating factor. MSF is also discussed in Schor et al (1994) In: Mammary Tumorigenesis and Malignant Progression, Kluwer Academic Publishers, Dickson, R. and Lippman, M. (eds).

Thus, MSF is believed to be produced by fibroblasts obtained from a majority of breast cancer patients and is not made by their normal adult

10

15

25

WO 99/31233 PCT/GB98/03766

counterparts. It is believed that measuring the levels of MSF, for example, in circulating blood or in serum or in urine, may be useful in identifying patients who have or are susceptible to cancer, or that it may be useful in prognosing the outcome of cancer. MSF producing fibroblasts are present in patients with a number of common epithelial tumours, such as carcinoma of the breast, lung and colon, as well as melanoma, and soft tissue sarcoma.

It is believed that it may be particularly useful to measure the levels of MSF in identifying patients who have or are susceptible to breast cancer, or in prognosing the outcome of breast cancer.

In addition, it is believed that MSF may be useful in wound healing since it is present in a majority of wound fluid samples. The directed migration of fibroblasts into the wound site and the transient increase in hyaluronic acid in granulation tissue during the wound healing response are both consistent with the involvement of MSF. (MSF stimulates the synthesis of a high molecular weight species of hyaluronic acid).

MSF is known to be related to fibronectin since certain antibodies raised to MSF also bind to fibronectin.

Fibronectin is a widely distributed glycoprotein present at high concentrations in most extracellular matrices, in plasma (300 µg/ml), and in other body fluids. Fibronectin is a prominent adhesive protein and mediates various aspects of cellular interactions with extracellular matrices including migration. Its principal functions appear to be in cellular migration during development and wound healing, regulation of cell growth and differentiation, and haemostasis/thrombosis.

Further progress in understanding MSF was hindered by the fact that it has not been clear whether MSF is a degradation or breakdown product of fibronectin, and because MSF appears to be structurally related to fibronectin.

We have now discovered that MSF is not a breakdown product of fibronectin but that it appears, quite unexpectedly, to be a "mini" splice variant of fibronectin. The amino acid sequence of MSF, disclosed for the first time herein, reveals unexpected regions of dissimilarity with fibronectin. This has led to previously unavailable methods of measuring, identifying and localising MSF becoming available. The availability of a polynucleotide encoding MSF, disclosed for the first time herein, makes available methods for producing MSF and useful variants thereof, and makes available new methods of specifically identifying, measuring and localising MSF.

A first aspect of the invention provides a recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

20

5

10

15

```
NLVATCLPVRASLPHRLN
    MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
    RQAQQMVQPQSPVAVSQSKPGCYDNGKHY
                                      CYGGSRG
        QQWERTYLGNALVCT
                                                    F
      E A E E T C F D K Y T G N T
D C T C I G A G R G R I S C
                                  Y R V
25
                                        GDT
                                              н Е
                                          R C
                                  T
                                      A N
                                                  GG
                            YMLEC
                                          LGNG
                                                  ΚG
                  H E T
                        GG
                                      V
                                        С
            R R
                Ρ
                  DHAAG
                            T S
                                Y V
                                    V
                                      GETWEKP
      IAEKCF
                GEGSGRIT
                                CTSRNRCND
      D C
          T C L
                                      CICTGNGRGE
                  IGDTWSKKDNRGNLL
30
                                                  Y
                                                      Ρ
      RHTSVQ
        PPYGHCVTDSGV
                                V
                                  YSVGMQW
                                                L
                                                  K
                                                      Q
      Ρ
                            Q
T
                                              G
          TCLGNGVS
                          С
                              Ε
                                T
                                  A V
                                      Τ
                                        Q
                                          T
                                            Y
                                                G
                                                  Ν
      L C
                              T
                                S
                                    Y
                                      Ε
                                                Y
                                                  S
                                                    F
                        D
                          S
                                  N
                                          D
                                            Q
                                              K
          F
            Т
              Y N D
                    R
                      T
                                        Q
        Р
                                        F
35
    V
                    N
                      S
                        N
                          G
                            A L
                                С
                                  Η
                                    F
                                      Ρ
                                          L
                                            YNNHN
        V
          QTRGG
                N M K W C G T T Q N
T N E G V M Y R I G
                                    Y D
                                        ADOKF
    S
      Ε
        G
          RRDNM
                                  \mathsf{G} \; \mathsf{D} \; \mathsf{Q} \; \mathsf{W} \; \mathsf{D} \; \mathsf{K} \; \mathsf{Q} \; \mathsf{H} \; \mathsf{D} \; \mathsf{M} \; \mathsf{G}
        E
          ICT
        CVGNGRGEWTCIAYSQLRDQCIVD
    N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
40
```

15

20

25

30

Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y

or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives.

Figure 2 shows the amino acid sequence encoded by the cDNA insert in pMSF1 $\alpha$  which contains the coding sequence for human migration stimulating factor (MSF). Preferably the amino acid sequence is based on that between the most N-terminal methionine and the most C-terminal stop codon (which are marked X). Thus, it is preferred if the polynucleotide encodes a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660 (ie. starting MLRGPG... as marked and encoding ...LGY as marked), or variants of fragments or fusions or derivatives thereof or fusions of said variants or fragments.

Throughout the specification where the term MSF is used, and the context does not indicate otherwise, it includes a polypeptide which has an amino acid sequence given in Figure 2 labelled pMSF1 $\alpha$  and, in particular, the amino acid sequence given between positions 19 and 660.

Amino acid residues are given in standard single letter code or standard three letter code throughout the specification.

It will be appreciated that the recombinant polynucleotides of the invention are not polynucleotides which encode fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a polynucleotide which encodes a portion or portions of MSF which are portions that distinguish

MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

The polynucleotide may be DNA or RNA but it is preferred if it is DNA.

The polynucleotide may or may not contain introns. It is preferred that it does not contain introns and it is particularly preferred if the polynucleotide is a cDNA.

A polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given in Figure 1. Thus, a polynucleotide of the invention includes the sequence

```
CAAACTTGGT GGCAACTTGC CTCCCGGTGC GGGCGTCTCT CCCCCACCGT
   CTCAACATGC TTAGGGGTCC GGGGCCCGGG CTGCTGCTGC TGGCCGTCCA
15
   GTGCCTGGGG ACAGCGGTGC CCTCCACGGG AGCCTCGAAG AGCAAGAGGC
   AGGCTCAGCA AATGGTTCAG CCCCAGTCCC CGGTGGCTGT CAGTCAAAGC
   AAGCCCGGTT GTTATGACAA TGGAAAACAC TATCAGATAA ATCAACAGTG
   GGAGCGGACC TACCTAGGCA ATGCGTTGGT TTGTACTTGT TATGGAGGAA
   GCCGAGGTTT TAACTGCGAG AGTAAACCTG AAGCTGAAGA GACTTGCTTT
20
   GACAAGTACA CTGGGAACAC TTACCGAGTG GGTGACACTT ATGAGCGTCC
   TAAAGACTCC ATGATCTGGG ACTGTACCTG CATCGGGGCT GGGCGAGGGA
   GAATAAGCTG TACCATCGCA AACCGCTGCC ATGAAGGGGG TCAGTCCTAC
   AAGATTGGTG ACACCTGGAG GAGACCACAT GAGACTGGTG GTTACATGTT
   AGAGTGTGTG TGTCTTGGTA ATGGAAAAGG AGAATGGACC TGCAAGCCCA
25
   TAGCTGAGAA GTGTTTTGAT CATGCTGCTG GGACTTCCTA TGTGGTCGGA
   GAAACGTGGG AGAAGCCCTA CCAAGGCTGG ATGATGGTAG ATTGTACTTG
   CCTGGGAGAA GGCAGCGGAC GCATCACTTG CACTTCTAGA AATAGATGCA
   ACGATCAGGA CACAAGGACA TCCTATAGAA TTGGAGACAC CTGGAGCAAG
   AAGGATAATC GAGGAAACCT GCTCCAGTGC ATCTGCACAG GCAACGGCCG
30
   AGGAGAGTGG AAGTGTGAGA GGCACACCTC TGTGCAGACC ACATCGAGCG
   GATCTGGCCC CTTCACCGAT GTTCGTGCAG CTGTTTACCA ACCGCAGCCT
   CTACTCTGTG GGGATGCAGT GGCTGAAGAC ACAAGGAAAT AAGCAAATGC
   TTTGCACGTG CCTGGGCAAC GGAGTCAGCT GCCAAGAGAC AGCTGTAACC
   CAGACTTACG GTGGCAACTC AAATGGAGAG CCATGTGTCT TACCATTCAC
    CTACAACGAC AGGACGGACA GCACAACTTC GAATTATGAG CAGGACCAGA
    AATACTCTTT CTGCACAGAC CACACTGTTT TGGTTCAGAC TCGAGGAGGA
    AATTCCAATG GTGCCTTGTG CCACTTCCCC TTCCTATACA ACAACCACAA
    TTACACTGAT TGCACTTCTG AGGGCAGAAG AGACAACATG AAGTGGTGTG
    GGACCACAC GAACTATGAT GCCGACCAGA AGTTTGGGTT CTGCCCCATG
    GCTGCCCACG AGGAAATCTG CACAACCAAT GAAGGGGTCA TGTACCGCAT
    TGGAGATCAG TGGGATAAGC AGCATGACAT GGGTCACATG ATGAGGTGCA
    CGTGTGTTGG GAATGGTCGT GGGGAATGGA CATGCATTGC CTACTCGCAG
    CTTCGAGATC AGTGCATTGT TGATGACATC ACTTACAATG TGAACGACAC
```

20

25

30

WO 99/31233 PCT/GB98/03766

ATTCCACAG CGTCATGAAG AGGGGCACAT GCTGAACTGT ACATGCTTCG
GTCAGGGTCG GGGCAGGTGG AAGTGTGATC CCGTCGACCA ATGCCAGGAT
TCAGAGACTG GGACGTTTTA TCAAATTGGA GATTCATGGG AGAAGTATGT
GCATGGTGTC AGATACCAGT GCTACTGCTA TGGCCGTGGC ATTGGGGAGT
GGCATTGCCA ACCTTTACAG ACCTATCCAA GCTCAAGTGG TCCTGTCGAA
GTATTTATCA CTGAGACTCC GAGTCAGCCC AACTCCCACC CCATCCAGTG
GAATGCACCA CAGCCATCTC ACATTTCCAA GTACATTCTC AGGTGGAGAC
CTGTGAGTAT CCCACCCAGA AACCTTGGAT ACTGAGTCTC CTAATCTTAT
CAATTCTGAT GGTTTCTTTT TTTCCCAGCT TTTGAGCCAA CAACTCTGAT
TAACTATTCC TATAGCATTT ACTATATTTG TTTAGTGAAC AAACAATATG
TGGTCAATTA AATTGACTTG TAGACTGAAA AAAAAAAAA AAAAAAAA

It is particularly preferred if the polynucleotide of the invention is one which comprises the polynucleotide whose sequence is given between positions 57 and 1982 in Figure 1 since this is believed to be the coding sequence for human MSF.

The invention includes a polynucleotide comprising a fragment of the recombinant polynucleotide of the first aspect of the invention. Preferably, the polynucleotide comprises a fragment which is at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length. Such polynucleotides are useful as PCR primers.

A "variation" of the polynucleotide includes one which is (i) usable to produce a protein or a fragment thereof which is in turn usable to prepare antibodies which specifically bind to the protein encoded by the said polynucleotide or (ii) an antisense sequence corresponding to the polynucleotide or to a variation of type (i) as just defined. For example, different codons can be substituted which code for the same amino acid(s) as the original codons. Alternatively, the substitute codons may code for a different amino acid that will not affect the activity or immunogenicity of the protein or which may improve or otherwise modulate its activity or immunogenicity. For example, site-directed mutagenesis or other

15

20

25

techniques can be employed to create single or multiple mutations, such as replacements, insertions, deletions, and transpositions, as described in Botstein and Shortle, "Strategies and Applications of *In Vitro* Mutagenesis," *Science*, **229**: 193-210 (1985), which is incorporated herein by reference. Since such modified polynucleotides can be obtained by the application of known techniques to the teachings contained herein, such modified polynucleotides are within the scope of the claimed invention.

Moreover, it will be recognised by those skilled in the art that the polynucleotide sequence (or fragments thereof) of the invention can be used to obtain other polynucleotide sequences that hybridise with it under conditions of high stringency. Such polynucleotides includes any genomic DNA. Accordingly, the polynucleotide of the invention includes polynucleotide that shows at least 55 per cent, preferably 60 per cent, and more preferably at least 70 per cent and most preferably at least 90 per cent homology with the polynucleotide identified in the method of the invention, provided that such homologous polynucleotide encodes a polypeptide which is usable in at least some of the methods described below or is otherwise useful. It is particularly preferred that in this embodiment, the polynucleotide is one which encodes a polypeptide containing a portion or portions that distinguish MSF from fibronectin.

It is believed that MSF is found in mammals other than human. The present invention therefore includes polynucleotides which encode MSF from other mammalian species including rat, mouse, cow, pig, sheep, rabbit and so on.

Per cent homology can be determined by, for example, the GAP program of the University of Wisconsin Genetic Computer Group.

DNA-DNA, DNA-RNA and RNA-RNA hybridisation may be performed in aqueous solution containing between 0.1XSSC and 6XSSC and at temperatures of between 55°C and 70°C. It is well known in the art that the higher the temperature or the lower the SSC concentration the more stringent the hybridisation conditions. By "high stringency" we mean 2XSSC and 65°C. 1XSSC is 0.15M NaCl/0.015M sodium citrate.

Polynucleotides which hybridise at high stringency are included within the scope of the claimed invention.

"Variations" of the polynucleotide also include polynucleotide in which relatively short stretches (for example 20 to 50 nucleotides) have a high degree of homology (at least 80% and preferably at least 90 or 95%) with equivalent stretches of the polynucleotide of the invention even though the overall homology between the two polynucleotides may be much less. This is because important active or binding sites may be shared even when the general architecture of the protein is different.

20

10

15

By "variants" of the polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter the activity of the said MSF.

Variants and variations of the polynucleotide and polypeptide include natural variants, including allelic variants and naturally-occurring mutant forms.

20

25

MSF may be assessed in bioassays based on its stimulation of adult skin fibroblast migration, for example, as is described in Picardo *et al* (1991) *The Lancet* 337, 130-133. Specificity for MSF may be inferred by neutralisation of migration stimulating activity by anti-MSF polyclonal antibodies (as herein disclosed). MSF may also be assayed using immunological techniques such as ELISA and the like.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

Such variants may be made using the methods of protein engineering and site-directed mutagenesis well known in the art.

Preferably, the variant or variation of the polynucleotide encodes a MSF that has at least 30%, preferably at least 50% and more preferably at least 70% of the activity of a natural MSF, under the same assay conditions.

By "fragment of MSF" we include any fragment which retains activity or which is useful in some other way, for example, for use in raising antibodies or in a binding assay, but which is not a fragment of MSF which could also be a fragment of fibronectin.

By "fusion of MSF" we include said MSF fused to any other polypeptide. For example, the said protein kinase may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of MSF, or it may be fused to some other polypeptide which imparts some desirable characteristics on the MSF fusion. Fusions to any

15

20

WO 99/31233 PCT/GB98/03766

variant, fragment or derivative of MSF are also included in the scope of the invention.

A further aspect of the invention provides a replicable vector comprising a recombinant polynucleotide encoding MSF, or a variant, fragment, derivative or fusion of MSF or a fusion of said variant, fragment or derivative.

A variety of methods have been developed to operably link polynucleotides, especially DNA, vectors for example to complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to The vector and DNA segment are then joined by the vector DNA. hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are

20

25

WO 99/31233

The state of the s

DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the DNA encoding the polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki et al (1988) Science 239, 487-491. This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art.

In this method the DNA to be enzymatically amplified is flanked by two specific primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

The DNA (or in the case of retroviral vectors, RNA) is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the DNA encoding the polypeptide constituting the compound of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the

polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

10

15

20

25

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance.

25

WO 99/31233 PCT/GB98/03766

Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

The vectors typically include a prokaryotic replicon, such as the ColE1 ori, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as E. coli, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical prokaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and pTrc99A and pKK223-3 available from Pharmacia, Piscataway, NJ, USA.

- A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.
- An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.
- Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *HIS3*, *TRP1*, *LEU2* and *URA3*. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Other vectors and expression systems are well known in the art for use with a variety of host cells.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research

Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic and kidney cell lines. Yeast host cells include YPH499, YPH500 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 and 293 cells which are human embryonic kidney cells. Preferred insect cells are Sf9 cells which can be transfected with baculovirus expression vectors.

Transformation of appropriate cell hosts with a DNA construct of the 15 present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen et al (1972) Proc. Natl. Acad. Sci. USA 69, 2110 and Sambrook et al (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring 20 Harbor, NY. Transformation of yeast cells is described in Sherman et al (1986) Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor, NY. The method of Beggs (1978) Nature 275, 104-109 is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome 25 formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA.

Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

For example, many bacterial species may be transformed by the methods described in Luchansky *et al* (1988) *Mol. Microbiol.* 2, 637-646 incorporated herein by reference. The greatest number of transformants is consistently recovered following electroporation of the DNA-cell mixture suspended in 2.5X PEB using 6250V per cm at 25μFD.

10

15

20

25

Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) Methods Enzymol. 194, 182.

Successfully transformed cells, ie cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant DNA, successful transformation can be confirmed by well known immunological methods when the recombinant DNA is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity.

15

20

25

Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium.

A further aspect of the invention provides a method of making MSF or a variant, derivative, fragment or fusion thereof or a fusion of a variant, fragment or derivative, the method comprising culturing a host cell comprising a recombinant polynucleotide or a replicable vector which encodes said MSF or variant or fragment or derivative or fusion, and isolating said MSF or a variant, derivative, fragment or fusion thereof of a fusion or a variant, fragment or derivative from said host cell.

Methods of cultivating host cells and isolating recombinant proteins are well known in the art. It will be appreciated that, depending on the host cell, the MSF produced may differ from that which can be isolated from nature. For example, certain host cells, such as yeast or bacterial cells, either do not have, or have different, post-translational modification systems which may result in the production of forms of MSF which may be post-translationally modified in a different why to MSF isolated from nature. It is preferred if the host cell is a non-human host cell; move preferably it is not a mammalian cell.

It is preferred that recombinant MSF is produced in a eukaryotic system, such as an insect cell.

A further aspect of the invention provides MSF or a variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative obtainable by the methods herein disclosed.

5 A further aspect of the invention provides a polypeptide comprising the amino acid sequence

```
NLVATCLPVRASLPHRLN
    MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
    RQAQQMVQPQSPVAVSQSKPGC
                                                    Y D
10
                                       T C
             WERTYLGNALVC
                                           Y
                                             GG
                                                 SR
      NQQWERTYLGNALV
EAEETCFDKYTGNT
                                           \mathsf{G} \mathsf{D} \mathsf{T}
                                                  YERP
                                     Y
                                       IANRCHEGG
                              I S C
                                     T
           Т
               ΙG
                   AGRGR
                              YMLEC
                                         V
                                           CLGNGKG
                   HETGG
             R R P
           EKCFDHAAGTSYV
                                       VGETWEKP
15
      ΙA
            \texttt{T} \ \texttt{C} \ \texttt{L} \ \texttt{G} \ \texttt{E} \ \texttt{G} \ \texttt{S} \ \texttt{G} \ \texttt{R} \ \texttt{I} \ \texttt{T} \ \texttt{C} \ \texttt{T} \ \texttt{S} \ \texttt{R} \ \texttt{N} \ \texttt{R} \ \texttt{C} \ \texttt{N} \ \texttt{D} \ \texttt{Q} \ \texttt{D} 
                                                          T
     V D
         С
     R I G D T W S K K D N R G N L L Q C I C T G N G R
E R H T S V Q T T S S G S G P F T D V R A A V Y Q
                                                          G
                                       s
V
                                           G M Q W L
Q T Y G G
     QPPPYGHCVTDSGV
                                   V Y
                                         V
                              Q E
T T
                                         Т
                                                      N S
    MLCTCLGNGVSC
                                   T A
20
                                   S N Y
                                         E
                                           Q
                                             D Q K
                                                    Y
                                                      SF
                                                          С
     VLPFTYNDRT
                          D S
                                L
                                           F
                                                YNNHN
               RGGNSNG
                              Α
                                   C H F P
                                             L
       L V
           Q
                                   QNYDADQKF
           RRDNMKWCGT
                                T
                                                      GFC
     SEG
               T T N E G V M Y R I G D Q W D K Q H D M G H M M R
     H E E
           I C
     CTCVGNGRGEWTCIAYSQLRDQ
                                                        V D
                                                    CI
25
     NVNDTFHKRHEEGHMLNC
                                           T
                                              С
                                                F G
                                                      G
                                                    Q
                                                      Y
     D P V D Q C Q D S E T G T F
                                   ΥQ
                                       I G D
                                             S
                                                W
                                                  Ε
                                                    K
                                                        V
                                                           Η
     QCYCŸGRGIGEWHC
                                                      SG
                                   Q
                                     P
                                       L
                                         Q
                                           T
                                              Y P S
                                                    S
                                                          Ρ
                              IQWNAP
                                           Q P S H I S K Y I L
             PSQPNSHP
         ΕT
     RPVSIPPRNLGY
30
```

or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

35 Thus, a polypeptide of the invention includes

```
NLVATCLPVRASLPHRLN
      MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
       R Q A Q Q M V Q P Q S P V A V S 
                                                 QSKPGC
                                                       Y
      INQQWERTYLGNALVC
                                                          GG
                                                                     G
                                                                        F N
40
                                                 T
                                                    С
                                                               S
                                                                  R
                                                 R V
                                                               Y
                                 Y
                                      G N
                                            T
                                               Y
                                                       GDT
                                                                  E
                                                                     R
              EETC
                         F
                           DΚ
                                    Т
      PEA
                                         s c
                                                  IANRCHE
                      GAGRGRI
                                               Т
              Т
                 С
                   I
                                                          LGNGKG
              WRRPHETGGYMLEC
                                                    V
                                                       С
           T
        IAEKCFDHAAGTSYVVGETWEKPY
               \texttt{T} \; \texttt{C} \; \texttt{L} \; \texttt{G} \; \texttt{E} \; \texttt{G} \; \texttt{S} \; \texttt{G} \; \texttt{R} \; \texttt{I} \; \texttt{T} \; \texttt{C} \; \texttt{T} \; \texttt{S} \; \texttt{R} \; \texttt{N} \; \texttt{R} \; \texttt{C} \; \texttt{N} \; \texttt{D} \; \texttt{Q} \; \texttt{D} \; \texttt{T} 
45
      V D C
                                                  QCICTGNGR
              DTWSKKDNRGNLL
           G
        R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P H P P P P Y G H C V T D S G V V Y S V G M Q W L K T Q G N K Q
```

20

30

35

MLCTCLGNGVSCQETAVTQTYGGNSNGE V L P F T Y N D R T D S T T S N Y E Q QΚ Y S F С T D LYN NΗ LCHFPF LVOTRGGNSN G A QNYDADQKF T G F C G TGRRDNMKW I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R Ε  $\verb|VGNGRGEWTCIAYSQLRDQCI| \\$ С V N D T F H K R H E E G H M L N C T C F G Q G R G YQIGDSWEKY  $\verb"VDQCQDSETGT" F"$ YCYGRGIGEWHCQPLQTYPSSSGP V TETPSQPNSHPIQWNAPQPSHISKYIL 10 VSIPPRNLGY

Preferably, the polypeptide comprises the amino acid sequence shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

It will be appreciated that the polypeptides of the invention are not fibronectin or fragments of fibronectin such as the gelatin binding domain. Preferably, the fragments and variants and derivatives are those that include a portion or portions of MSF which are portions that distinguish MSF from fibronectin and which are described in more detail below and by reference to Figure 2.

25 Preferably, the polypeptide of the invention is one which has migration stimulating factor activity.

Further aspects of the invention provide antibodies which are selective for MSF (and do not cross react with fibronectin) and antibodies which are selective for fibronectin (and do not cross react with MSF).

By "selective" we include antibodies which bind at least 10-fold more strongly to one polypeptide than to the other (ie MSF vs fibronectin); preferably at least 50-fold more strongly and more preferably at least 100-fold more strongly.

Such antibodies may be made by methods well known in the art using the information concerning the differences in amino acid sequence between MSF and fibronectin disclosed herein. In particular, the antibodies may be polyclonal or monoclonal.

Suitable monoclonal antibodies which are reactive as said may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", SGR Hurrell (CRC Press, 1982). Polyclonal antibodies may be produced which are polyspecific or monospecific. It is preferred that they are monospecific.

One embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
                LLLAVQCLGTAVPSTGASKSK
   MLRGPGPGL
                Q S P V A V S Q S K P G C Y D N G K H Y
20
               Ρ
   RQAQQM
            V
             Q
               YLGNALVCTCYGGSRGFNCESK
       QWERT
   PEAEETCFDKYTGNTYRVGDTYERPKDSMI
          IGAGRGRISCTIANRCHE
                                     GGQ
       T C
   GDTWRRPHETGGYMLEC
                            V C
                               LGN
                                     Κ
    I A E K C F D H A A G T S Y
D C T C L G E G S G R I T C
                           V
                               TWEK
                                     P
                         V
                            GE
25
                        C T S
                               RCND
                            R N
                                     Q
          WSKKDNR
                     G N
                        LLQC
                              I
                               С
                                 T
                                  G N
       D
         T
          VQTTSSGSGPF
                           TDVRAAV
    RHT
         S
    PPPYGHCVTDSGVVYSVGMQWLKT
   MLCTCLGNGVSCQETAVTQTYGG
                                     N
30
         TYNDRTDSTTSNY
                            E Q D Q K
                                     S
      PF
   V L V Q T R G G N S N G A L C
                         H F
                            Ρ
                               Ŀ
                                  Ν
                                     Н
                           Y
                                        C P
                       Т
                        Q
                            DΑ
                               D
                                 Q
                                  K F
                                     G F
   SEGRRDNMKW
                  С
                   G
                     T
                         N
                     Y
                         GDQWDK
                                  Q H D M G H
                      RΙ
                 G V
                   Μ
        I C
           T
            T
              ΝE
35
         GNGRGEWT
                     С
                      IAYS
                            Q
                              LRDQ
                                    С
        V
        DTFHKRHEEGHMLNCTCFG
      N
   D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G
                                           V
      YCYGRGIGEWHCQPLQTYPSSSGPVE
   I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
40
   RPVSIPPRNLGY
```

or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.

A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 $\alpha$  or natural variants thereof but which epitope is not present in fibronectin.

A further embodiment provides an antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

15 NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVQCLG TAVPSTGASKSK QSKPGCYDNGKHY  $R Q A Q Q M V Q P Q S P V A \overline{V} S$ I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N GNTYRVGDTYERPKDSMI FDKY T PEAEE С IGAGRGRISCTIANRCHEGGQSYK С T WRRPHETGGYMLECVCLGNGKGEWT IAEKCFDHAAGTSYVVGETWE Y ΚP G V D C T C L G E G S G R I T C T S R N R C N D RIGDTWSKKDNRGNLL Q C I C T G N G R 25 V A A V Y E R H T S V Q T T S S G S G R P F Т D Y GMQWLKT  ${\tt T}$  D S G V V S V QPPPYGHC V V S C Q E T A V Т QT YGGNSN TCLGN G MLC QDQKYSF T D S T T SNYE Y N D R V L P T C H F P F L Y N N H NQTRGGNSNGAL 30 V L V S E G R R D N M K W C G T T Q N Y D A D H E E I C T T N E G V M Y R I G D Q W D K F DΩ K QH D M G I V D D CVGNGRGEWTC Ι Α Υ S Q L R D Q С GRGRWKC NVNDTFHKRHEEGHMLNC Τ С F G Q G T F Y Q I G D S W E K Y V H G  $\mathbf{T}$ 35 0 C ODSE YGRGIGEWHCQPLQTYPSSSGPVEVF PSQPNSHPIQWNAPQPSHISKYILRW T Ε RPVSIPPRNLGY

between positions 19 and 660 or natural variants thereof but which is epitope is not present in fibronectin.

10

WO 99/31233

PCT/GB98/03766

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

ISKYILRWRPVSIPPRNLGY; or

QQWERTYLGNALVCTCYGGSR; or

PCVLPFTYNDRTDSTTSNYEQDQ; or

TDHTVLVQTRGGNSNGALCH; or

VGNGRGEWTCIAYSQLRDQCI

which are found in MSF. The underlined amino acid(s) indicate the difference between MSF and fibronectin.

These peptides contain and flank regions of difference in amino acid sequence between MSF and fibronectin as shown in Figure 2 which are believed to be useful in distinguishing MSF and fibronectin using antibodies.

A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 or natural variants thereof.

20

15

A further embodiment provides an antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 between positions 19 and 660 or natural variants thereof.

25

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVQCLGTAVPSTGASKSK RQAQQMVQPQSPVAV SQSKPGC Y D N G КН С INQQWERTYLGNALV С GGSRG F N C T Y RVGDTYERPKD PEAEETCFDKYTGNT Y 5 DCTCIGAGRGRISC T IANRCHEGG D T W R R P H E T G G Y M L E C V C L G N G K G E F D H A A G T S Y V V G E T W E K P Y Q G W M M AEKC C L G E G S G R I T C T S R N R C N D Q D С T G IGDTWSKKDNRGNLLQ С ΙC T N 10 RHTSVQTTSSGSGPF D R A Α V Ρ PYGHCVTDSG V V Y S V GMQWLK Ρ A V GGNS QET T Y TCLGNGVS Q T С С YNDRTDSTT SNYE QDQKYSF Y N N H N Y SNGALCHFP F L RGGN T 15 V QNYDADQKFGFCP GRRDNMKWCGTT Ε TTNEGVMYRIGDQWDKQHDMGH I C H E E CVGNGRGEWTCIAYSQLRDQC NVNDTFHKRHEEGHMLNC T C F GΩ GRGR D P V D Q C Q D S E T G T F Y QIG D S W ΕK Y V H G 20 QPLQTYPSSSGPVEVF CYCYGRGIGEWHC TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

or natural variants thereof.

A further embodiment provides an antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660 or natural variants thereof.

It is particularly preferred if the antibody is reactive towards a molecule comprising any one of the peptides:

QQWERTYLGN<u>V</u>LVCTCYGGSR or

EPCVLPFTYN<u>G</u>RT<u>FYSCTTEGRQDGHLWC</u>STTSNYEQDQ or

CTDHTVLVQTQGGNSNGALCH or

VGNGRGEWTC<u>Y</u>AYSQLRDQCI or

ISKYILRWRP<u>KNSVGRWKEA</u> or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2. The underlined amino acid(s) indicate the difference between fibronectin and MSF.

10

15

These peptides themselves may be useful for raising antibodies, but selective antibodies may be made using smaller fragments of these peptides which contain the region of difference between MSF and fibronectin.

Peptides in which one or more of the amino acid residues are chemically modified, before or after the peptide is synthesised, may be used providing that the function of the peptide, namely the production of specific antibodies in vivo, remains substantially unchanged. modifications include forming salts with acids or bases, especially physiologically acceptable organic or inorganic acids and bases, forming an ester or amide of a terminal carboxyl group, and attaching amino acid protecting groups such as N-t-butoxycarbonyl. Such modifications may protect the peptide from in vivo metabolism. The peptides may be present as single copies or as multiples, for example tandem repeats. tandem or multiple repeats may be sufficiently antigenic themselves to obviate the use of a carrier. It may be advantageous for the peptide to be formed as a loop, with the N-terminal and C-terminal ends joined together, or to add one or more Cys residues to an end to increase antigenicity and/or to allow disulphide bonds to be formed. If the peptide is covalently linked to a carrier, preferably a polypeptide, then the arrangement is preferably such that the peptide of the invention forms a loop.

25

20

According to current immunological theories, a carrier function should be present in any immunogenic formulation in order to stimulate, or enhance stimulation of, the immune system. It is thought that the best carriers embody (or, together with the antigen, create) a T-cell epitope. The

15

20

WO 99/31233 PCT/GB98/03766

peptides may be associated, for example by cross-linking, with a separate carrier, such as serum albumins, myoglobins, bacterial toxoids and keyhole limpet haemocyanin. More recently developed carriers which induce T-cell help in the immune response include the hepatitis-B core antigen (also called the nucleocapsid protein), presumed T-cell epitopes Thr-Ala-Ser-Gly-Val-Ala-Glu-Thr-Thr-Asn-Cys, betasuch as galactosidase and the 163-171 peptide of interleukin-1. The latter compound may variously be regarded as a carrier or as an adjuvant or as both. Alternatively, several copies of the same or different peptides of the invention may be cross-linked to one another; in this situation there is no separate carrier as such, but a carrier function may be provided by such cross-linking. Suitable cross-linking agents include those listed as such in Pierce catalogues, for example glutaraldehyde, Sigma and carbodiimide and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1carboxylate, the latter agent exploiting the -SH group on the C-terminal cysteine residue (if present).

If the peptide is prepared by expression of a suitable nucleotide sequence in a suitable host, then it may be advantageous to express the peptide as a fusion product with a peptide sequence which acts as a carrier. Kabigen's "Ecosec" system is an example of such an arrangement.

The peptide of the invention may be linked to other antigens to provide a dual effect.

25

A further aspect of the invention provides a method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

N L V A T C L P V R A S L P H R L N

30 M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K

RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ CYGGSRG YLGNALVCT Ν ERT F D K Y T G N T Y R V G D T Y E R P K D С GAGRGRISCTIANRCHEGG Ι DTWRRPHETGGYMLEC VCLGNG I A E K C F D H A A G T S Y V V G E T Ε С D D V D C T C L G E G S G R I T С Т S R N R N G N G R RIGDTWSKKDNRGN L L Q С I C T T D V R A A V Y GSGPF  $R \ H \ T \ S \ V \ Q \ T \ T$ S S v G V V Y S VGMQWLKT T S 10 Y GН С D PΡ Q E T A V GNSN LGNG v s С T Q T Y G С Τ С TSNYEQDQKYS YNDRTDS T Ρ F Т LYNNHN TRGGNSNGALCHFPF V Q R R D N M K W C G T T Q N Y D A D Q K I C T T N E G V M Y R I G D Q W D K Q F G M G QH D 15 L R D Y S С Ι V VGNGRGEW Т Α Q С ĞΩ HMLNC T C F G TFHKRHEE V N D IGDSWEK DSETG T F Y Q С Q Q T Y P S S S G P V E V F RGIGEWHCQP L Y G С IQWNAPQPSHISKYILRW E T P S Q P N S H P 20 RPVSIPPRNLGY

or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin. Suitable peptides are disclosed above.

A still further aspect of the invention provides a method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLL L TAVPSTGASKSK Α V Q C G KPGCYDN V S 35 RQAQQMVQP Q S ΡV Α Q S CYGGSRG GNALVC  $\mathbf{T}$ Q A QW ERTY L V G D T Y E R P F D K Y TGNT ΥR С T E E GAGRGRISCT IANRCHEGG С T С Ι WRRPHETGGYMLEC V N С L G C F D H A A G T S Y L G E G S G R I T C K P G 40 AEK V V Ε W Ε Т S R N R C N DQ C L G E GС L G N L Q С I C T G N G Đ R G K K Ν S G S G P F T D V R A A V Y Τ S Т S V Q Т Η G M Q W L K T Q T Y G G N S Y S V Y G H C V T D S G V V Ρ Ρ QETAVT GNGVSC 45 С T С L TYNDRTDSTTSNYE QDQKYS LPF LVQTRGGNSNGALC Н F Ρ F L Y N Ν Η N A D Q K Y D F G F EGRRDNMKW С G T T Q N GDQWDKQHDMG Y I T ΝE G V M R I C T V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y D T F H K R H E E G H M L N C T C F G Q G R G R W K C 50

```
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

5

10

15

20

25

30

or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF. Suitable peptides are disclosed above.

It will be appreciated that, with the advancements in antibody technology, it may not be necessary to immunise an animal in order to produce an antibody. Synthetic systems, such as phage display libraries, may be used. The use of such systems is included in the methods of the invention.

Before the present invention it was not possible to make use of the differences in amino acid sequence between fibronectin and MSF in order to make antibodies which are useful in distinguishing MSF and fibronectin since it was not known that MSF and fibronectin had significant differences in structure or what those differences were. As is discussed in more detail below, such antibodies are useful in cancer diagnosis. It will also be appreciated that such antibodies which distinguish MSF and fibronectin are also useful research reagents. Suitably, the antibodies of the invention are detectably labelled, for example they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the antibodies are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or they may be linked to an enzyme. Typically, the enzyme is one which can convert a non-coloured (or non-fluorescent) substrate to a coloured (or fluorescent) product. The antibody may be labelled by biotin (or streptavidin) and then detected indirectly using

streptavidin (or biotin) which has been labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or the like or they may be linked to an enzyme of the type described above.

It is particularly preferred if peptides are made, based on the amino acid sequence of MSF and fibronectin, which allow for specific antibodies to be made.

Thus, a further aspect of the invention provides a molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
15
   RQAQQMVQPQSPVAVSQSKPGCYDN
                                                G K
    I N Q Q W E R T Y L G N A L V C T C Y G G S R G F
    PEĀĒETCFDKYTGNT
                              Y R V
                                    G
                                         Υ
                                           E R P
                                                K
                                   NRC
                            С
                                         ΗE
                                             GG
   WDCTCIGAGRGRI
                           S
                              T
                                Ι
                                  Α
                         Y
                                C
                                  V
                                    C L
                                       G
                                         N
                                           G
                                             K
                                              G
20
    GDTWRRPHE
                   TGG
                           Μ
                             L
                              Ε
                                V
                                  GETWE
                                           K
                         T S Y
                              V
     IAEKCFDHAAG
           CLGEGSGRIT
                             CTSRNRCNDQDT
    V
     D
       С
         T
                                       TGNG
                                               R
         DTWSKKDNRGNLL
                                Q
                                  С
                                    ΙC
    RΙ
       G
                                T D V R A A V
                                             Y
                  TSSGSGPF
    ERHTSVQT
    Q P P P Y G H C V T D S G V V Y S V G M Q W
                                           L
                                             K
25
                                               S
           \texttt{C} \;\; \texttt{L} \;\; \texttt{G} \;\; \texttt{N} \;\; \texttt{G} \;\; \texttt{V} \;\; \texttt{S} \;\; \texttt{C} \;\; \texttt{Q} \;\; \texttt{E} \;\; \texttt{T}
                              A V
                                  T
                                    0
                                      T
                                         G
                                           G
                                             Ν
       СТ
                                Y
                                       QKY
                                             S
                                              F
    V L P F T Y N D R T D
                         Т
                       S
                           T
                             S
                              N
                                  Ε
                                    Q
                                      D
                               H F
                                  Р
                                    F
                                        Y
                                         NNHNY
                       G
                         Α
                           L
                             С
                                      L
    VLVQTRGGN
                    s N
                         Т
                           \mathbf{T}
                             QN
                                YDADQKF
       GRRDNMKWC
                       G
                                         QHDMGHMMR
                  EGVMYRI
                                  QWDK
                               G D
30
             Т
              Т
                N
                                           CIVDD
              GRGEWTC
                           IAYS
                                  QLRDQ
       С
         V
           G N
      V N D T F H K R H E E G H M L N C T C F G Q G R G R
                                               V
                                                  G
              Q D S E T G T F Y Q I G D S W
                                         E
       VDQC
           YGRGIGEWHCQPLQT
                                      Y P
                                         S
                                           S
                                             SGPV
                                                    Ε
       Y C
                         IQWNAPQPSHISKYILRW
    ITETPSQPNSHP
35
    RPVSIPPRNL
```

or natural variants thereof but not reactive towards fibronectin.

A still further aspect of the invention provides a molecule which is capable of, following immunisation of an animal if appropriate, giving rise to

antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

NLVATCLPVRASLPHRLN LGTAVPSTGASKSK MLRGPGPGLLLLAV С Q SQSKPGCYDN Q S P V A V GKHY OAOQMV QP G N A L V C Т CYGGSRGF ERT Y L QW CFDKYTGNTYRVGDTYERPKDS EAEET IGAGRGRISCTIANRCHEGG T C D C G WRRPHETGGYMLE CVCLGNGK Т IAEKCFDHAAGTSYVVGETWEKP 10 CLGEGSGRITCTSRNRC N D  $\mathbf{T}$ G GDTWSKKDNRGNL L С Ι С T G N G R Q RI RAAV Y Ρ P F T D V ERH TSVOTTSSGSG V S GMQWL K Q G V Y V С T D S GH V Р Ρ Y Τ NG V s c QΕ Т A V T Q TY G G 15 С L G DRTDS Y S Т Y N  $\mathbf{T}$ T SNYE Q DQK Ρ F FLYNNH C H F P GNSNGAL V QTRG R R D N M K W C G T T Q N Y D A D Q K F I C T T N E G V M Y R I G D Q W D K Q H GF G D VGNGRGEWTC ΙA Y S Q L R D Q С Ι V D 20 С  $\mathbf{T}$ F GRGR ΝV NDTFHKRHEE G H M L N С T С G IGDS EKYVH F ΥQ W SET G Τ VDOC O D YGRGIGEWHCQPLQTYPSSSGP С PSQPNSHPIQWNAPQPSHISKYILRW Ε T 25 VSIPPRNLGY

or natural variants thereof.

The molecule is preferably a peptide but may be any molecule which gives rise to the desired antibodies. The molecule, preferably a peptide, is conveniently formulated into an immunological composition using methods well known in the art.

The peptides disclosed above form part of these aspects of the invention.

35

40

30

Peptides may be synthesised by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lu et al (1981) J. Org. Chem. 46, 3433 and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is effected using 20% piperidine in N,N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl

15

20

25

esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalising agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are N,N-dicyclohexyl-carbodiimide/1reversed using added a hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid Scavengers commonly used are containing a 50% scavenger mix. ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesised. Trifluoroacetic acid is removed by evaporation in vacuo, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 2QJ, UK. Purification may be effected by any one, or a combination of, techniques

10

such as size exclusion chromatography, ion-exchange chromatography and (principally) reverse-phase high performance liquid chromatography. Analysis of peptides may be carried out using thin layer chromatography, reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis.

It is now possible to make polynucleotides which can distinguish MSF and fibronectin and such polynucleotides are believed to be useful in the diagnosis and prognosis of cancer.

A further aspect of the invention provides a polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

15 NLVATCLPVRASLPHRLN QCLGTAVPSTGASKSK M L R G P G P G L L L L A VQ P Q S P V A V S Q S K P G C Y D N G K H Y Q Q Q M V Q W E R T Y L G N A L V C T C Y G G S R G F N C PEAEETCFDKYTGNTYRVGDTYERP 20 CIGAGRGRISCTIANRC H E GG N G G Ε С V С L K GDTWRRPHETGGYMLE Ρ Υ IAEKCFDHAAGTS GET WE K Y V V N R C N С D Q D DCTCLGEGSGR I T T S R Τ G N R KKDNRGN L С ΙC L Q 25 G DTWS T S S G S G P F DVRAAV Y V Q Τ S T R H V T D S G V V Y S V G M Q W L K T YGHC P P Ρ CLGNGVSCQETAVTQTŸG G N С T TYNDRTDSTTSNY E Q D QK NNH N VQTRGGNSNGALC Н F Ρ F 30 K F G F SEGRRDNMKWC G Т Т Q Ν Y DADQ YRĨGD QWDK QHDMG С Т NEG V Μ I T QCIVDD T IAYS QLRD G N G RGEW С N D T F H K R H E E G H M L N C T C F G Q G R G R V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G 35 C Y C Y G R G I G E W H C Q P L Q T Y P S S G PV T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W RPVSIPPRNLGY

or a natural variant thereof and a polynucleotide which encodes fibronectin.

A still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

5 NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVQCLGTAVPSTGASKSK Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K E A E E T C F D K Y T G N T Y R V G D T Y E R P K D S M I 10 WDCTCIGAGRGRISCTIANRCHE G D T W R R P H E T G G Y M L E C P I A E K C F D H A A G T S Y V V V C G E LGNGKG I A E K C F D H A A G T S Y V D C T C L G E G S G R I T C T T W ΕK Ρ Y RNRCN S D Q D ICTGN WSKKDNRGNLL Q С 15 T S V Q T T S S G S G P F T D V R A A V Y Q P Y G H C V T D S G V V Y S V G M Q W L K T P P CTCLGNGVSCQETAVTQTYGGNSN V L P F T Y N D R T D S T T S N Y E Q D Q K Y S V L V Q T R G G N S N G A L C H F 20 Q I YDAD Q ΚF F С SEGRRDNMKWCGT T N G QWDK QHDMG TTNEGVMY R G D HEEIC VGNGRGEWT С IAYSQLRDQ CI F H K R H E E G H M L N C T C F G Q G R G R W V N D Τ CQDSETGTFYQIGDSWEKYVHG 25 D P V D Q YGRGIGEWHCQPLQTYPSSSGPVEVF QCYC TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

30 or a natural variant thereof.

A yet still further aspect of the invention provides a polynucleotide which is capable of hybridising to a polynucleotide which encodes the polypeptide whose sequence is

35

```
NLVATCLPVRASLPHRLN
                           V
                            Q
                              CLGTAVPSTGASKSK
   MLRGPGPGLLLLA
                                QSKPGCYDNGKHY
                 P Q S P V A V S Q
Y L G N A L V C T
          Q M V
          WERT
                                  CYGGSRGFNC
   P E Ā Ē E T C F D K Y T G N T Y R V G D T Y E R P K D S M I
          CIGAGRGRISCTIANRCHE
                                             GG
         Т
     DTWRRPHETGGYMLEC
                                  VCLGNG
                                             K
     I A E K C F D H A A G T S Y
D C T C L G E G S G R I T C
                                             Р
                               V
                                V
                                  G E
                                      T W
                                          Ε
                                           K
                               Т
                                        C N D
                                S
                                      R
   VDCTCLGEGSGRI
                                  R N
            WSKKDNRGNL
                               L
                                             G
                                Q
                                  С
                                    I C T
                                          G N
     I G D T
           S
                  T S S G S
                           G P F
                                Т
                                  DVRAAV
            V
     R H T
              QΤ
                           V V Y S V G M Q W L K
                                                 Q
          Y G H C
                  VTDSG
     PPP
   MLCTCLGNGVSCQETAVTQTŸGG
                                             N S
                                                 N
   V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T
50
```

20

25

30

WO 99/31233 PCT/GB98/03766

```
S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
                         QWDKQHDMGHMMR
HEEICTTNEGV
                   RIGD
                M Y
                         QLRDQCIVDDI
CTCVGNGRGEWTCIAYS
        HKRHEEGHMLN
                         CTCFGQGRGRWKC
NVNDTF
  V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
     Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
 С
  Y C
 TETPSQPNSHPIQWNAPQPSHISKYILRW
  VSIPPRNLGY
```

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

Such polynucleotides can be designed by reference to Figures 1 and 2 and the known sequence of fibronectin (Kornblihtt et al (1985) EMBO J. 4, 1755-1759), and may be synthesised by well known methods such as by chemical synthesis or by using specific primers and template, a DNA amplification technique such as the polymerase chain reaction. The polynucleotide may be any polnucleotide, whether DNA or RNA or a synthetic nucleic acid such as a peptide nucleic acid, provided that it can distinguish polynucleotides which encode MSF and fibronectin as said. It is particularly preferred if the polynucleotide is an oligonucleotide which can serve as a hybridisation probe or as a primer for a nucleic acid Thus, the polynucleotide of this aspect of the amplification system. invention may be an oligonucleotide of at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length.

It is particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes MSF but does not hybridise to a mRNA (or cDNA) which encodes fibronectin.

It is also particularly preferred that the polynucleotide hybridises to a mRNA (or cDNA) which encodes fibronectin but does not hybridise to a

11718

199 1199 17

20

WO 99/31233 PCT/GB98/03766

mRNA (or cDNA) which encodes MSF. The nucleotide sequence of MSF cDNA is disclosed herein and the nucleotide sequence of fibronectin is known (for example, see Kornblihtt *et al* (1985) *EMBO J.* 4, 1755-1759). The skilled person can readily design probes which can distinguish MSF and fibronectin mRNAs and cDNAs based on this information. Differences between MSF and fibronectin include a 45 bp deletion from the first type II fibronectin repeat module in MSF, and the unique tail present in MSF.

Preferably, the polynucleotides of the invention are detectably labelled. For example, they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the polynucleotides are labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety or some other suitable detectable moiety. The polynucleotides may be linked to an enzyme, or they may be linked to biotin (or streptaridin) and detected in a similar way as described for antibodies of the invention.

A further aspect of the invention provides a method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
   RQAQQMVQPQSPVAVS
                             QSKP
                                       Y
                                         D
                                            GKH
                                         G
   INQQWERT
                YLGNA
                        L
                          V
                           С
                               С
                                Υ
                                  GG
                                     S
                                       R
25
                       GNTY
                             R V
                                GDT
                                     Y
                                       ERPKD
                  ΚY
                     Т
        EΕ
           T
             С
              F
                D
                             IANRCHE
              AGRGRIS
                          C T
                                         G
          С
           I
             G
                  TGGYMLEC
                               V
                                C
                                  L
                                    GNGK
        WRRPHE
      T
                        SYVVGETWEKPY
        EKCFDHAAGT
      Α
           LGEGSGRITCTSRNRCND
                                         Q D
30
   V D
        T
      GDTWSKKDNRGNLLQC
                                I
                                  С
                                    Т
                                     G
                                       N
                                         Y
                                    Α
                                       V
                  S
                       S
                        G
                          P
                            F
                             \mathbf{T}
                               D
                                 V
                                  R
                                     Α
     R H T S V Q T
                T
                    SG
                          V
                            Y
                             S
                                 G M
                                    Q
                                     W
                                       L
                                         K
                                          T
           GHC
                     S
                       G
                        V
                               V
     ΡP
          Y
                V
                  T
                    D
        Ρ
                  V
                       QE
                                    Y
                                       G N
           L G N G
                    S
                     С
                          Т
                            Α
                             V
                               T
                                Q
                                  T
                                     G
      С
        T
          С
                   DS\widetilde{T}
                                  D Q K Y S F C
L Y N N H N Y
                        T
                          S N
                             YEQD
35
       P
        F
          Т
           YNDRT
     L
                          CHF
                                F
           RGGNS
                   NGAL
                               Ρ
       V
        Q
          Т
                                          F
      GRRDNMKWCGTTQNYDADQKF
                                         G
    H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
     T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y
```

N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

NLVATCLPVRASLPHRLN 15 CLGTAVPSTGASKSK MLRGPGPGLLLLAV Q Q S P V A V S Q S K P G C Y D N G K H Y RQAQQMVQP QWERTYLGNALVCTCYGGSRGFNCESK F D K Y T G N T Y R V G D T Y E R P K D S M EETC IGAGRGRISCTIANRCHEGGQS 20 CTC WD G D T W R R P H E T G G Y M L E C V C L G N G K G E W I A E K C F D H A A G T S Y V V G E T W E K C L G E G S G R I T C T S R N R C N D Q D RIGDTWSKKDNRGN TGNGR G L L Q С I C TSSGSGPF T D VRAAVY Q P  $\hbox{\tt E} \hbox{\tt R} \hbox{\tt H} \hbox{\tt T} \hbox{\tt S} \hbox{\tt V} \hbox{\tt Q} \hbox{\tt T}$ 25 V T D S G V V Y S V G M Q W L K T QPPPYGHC LGNGVSCQETAVTQTYGGNSN Τ C YNDRTDSTTSNYEQDQKY F T Ρ V Q T R G G N S N G A L C H F P F L Y N N H N Y V L  $\begin{smallmatrix} g \end{smallmatrix} \begin{smallmatrix} \bar{R} \end{smallmatrix} R \begin{smallmatrix} D \end{smallmatrix} N \begin{smallmatrix} M \end{smallmatrix} K \begin{smallmatrix} W \end{smallmatrix} C \begin{smallmatrix} G \end{smallmatrix} T \begin{smallmatrix} T \end{smallmatrix} Q \begin{smallmatrix} N \end{smallmatrix} Y \begin{smallmatrix} D \end{smallmatrix} A \begin{smallmatrix} D \end{smallmatrix} Q \begin{smallmatrix} K \end{smallmatrix} F$ F 30 H E E I C T T N E G V M Y R Ï G D Q W D K Q H D M G I V D CVGNGRGEWT ΙA Y S QLRDQC С C T C F G Q GRGRWKC NVNDTFHKRHEEGHMLN YQIGDSWEKYVHG T F D P V D Q C Q D S E Т G IGEWHCQPLQTYPSSSGPVEVF 35 YGRG Y C PSQPNSHPIQWNAPQPSHISKYILRW Т RPVSIPPRNLGY

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising

35

40

WO 99/31233 PCT/GB98/03766

detecting in a sample from the patient the presence of a polypeptide whose sequence is

NLVATCLPVRASLPHRLN 5 M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K RQAQQMVQPQSPVAVS QSKPGCYD QQWERTYLGNAL V C T С Y GGSR CFDKYTGNTYRVGDT YERP ΚD PEAEET CIGAGRGRISCTIANRCHEGG CT10 G D T W R R P H E T G G Y M L E C V C L G N G K G PIAEKCFDHAAGTSYVVGETWEKP V D C T C L G E G S G R I T C T S R N R C N D Q D T R I G D T W S K K D N R G N L L Q C I C T G N G R E R H T S V Q T T S S G S G P F T D V R A A V Y Q G VTDSGV V Y S V 15 YGHC GMQWLK QPP С CLGNGVSCQETAVT Q Y GGNS N M L T T YNDRTDSTT SNYE QDQKYSF VLPFT C V Q T R G G N S N G A L C H F P F L Y N N G R R D N M K W C G T T Q N Y D A D Q K F YNNHN GF 20 HEEICTTNEGVMYRIGDQWDKQHDMG YSQLRDQC CVGNGRGEWTC ΙA GHMLNC T NDTFHKRHEE G R QDSETGTF Y QIGDSWEKY V D Q C V Η YGRGIGEWHCQPLQTYPSSSGP QCYC 25 ITETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

Preferably, the reagent which can distinguish MSF from fibronectin is an antibody as disclosed herein. The use of antibodies to detect specific polypeptides in samples is well known. For example, they can be used in enzyme-linked immunosorbend assays (ELISA) or they may be used in histopathological analysis. It is believed that the presence of MSF indicates an elevated risk of cancer.

MSF may be conveniently measured in suitable body fluids such as serum or urine, or in extracts of tissue, or in the medium used to culture patient derived cells *in vitro*.

15

20

The measurement of MSF is believed to be useful in a number of cancers as discussed above. Antibodies may be used to detect MSF in tissue sections by immunolocalisation. Sub-populations of MSF-producing fibroblasts are present in the normal adult (Irwin et al (1994) J. Cell Science 107, 1333-1346; Schor et al (1994) pp 277-298 in Mammary Tumorigenesis and Malignant Progression, Dickson, R. and Lippman, M. (eds), 1994, Kluwer Academic Publishers.

It will be appreciated that, as well as the MSF polypeptide being measured using the methods described herein in diagnosis or prognosis or determination of susceptibility to cancer, it may be desirable to detect MSF mRNA in a suitable sample or it may be desirable to detect any changes in the fibronectin gene which are associated with the production of MSF. Mutations in the MSF cDNA or fibronectin gene may be detected using methods well known in the art.

Thus, a further aspect of the invention provides a method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                         Q C L G T A V P S T G A S K S K
   MLRGPGPGLLLLAV
                                 PGCYDNGKH
   RQAQQMVQPQSPVAVS
                             Q S
                                K
   INQQWERTYLGNALVC
                             T C
                                YGGSRGFN
25
                      GNTYRV
                                GDTYERPKD
           T C
                 ΚY
                     T
        ΕE
              F
                D
                             IANRCHEGG
                      ISCT
           ΙG
                 RGR
          С
              ΑG
                     \mathsf{G} \mathsf{Y} \mathsf{M} \mathsf{L} \mathsf{E} \mathsf{C}
                              V
                                CLGNGK
                                         G
              ΗE
                  T G
         R R P
        M
                        S Y V V G E T W E K P
              D H A A G T
        EKC
             F
     ΙA
                  SGRITCTSRNRCND
30
           L G
              ΕG
   V D C
        Т
          С
                                I
V
                                   T
A
   RIGDTWSKKDNRGNLLQ
                              C
                                  С
                                     G N
                                        Y
                                      V
     RHTSVQTTS
                   S G S
                        G
                          Ρ
                           F
                             \mathbf{T}
                               D
                                  R
                                     Α
                          V
                           Y
                             S
                                GMQW
                                      L
                      G
                        V
                               V
     PPPYGH
              С
                V
                  T
                   D
                     S
                  V
                     С
                                   YGGN
              ΝG
                   S
                      Q
                        Ε
                          T
                           Α
                             V
                               T
                                Q
                                  Т
        T C
           L
             G
     L C
                     s T
                        T S N
                             YEQD
                                   QKYS
           YNDRT
                   D
35
       Ρ
        F
          \mathbf{T}
                                  LYNNHNY
           RGGNSNGAL
                          CHFPF
   V
       V
        Q
          Т
     EGRRDNMKWCGTTQNYDADQKFG
                                         F
    S
    HEEICTTNEGVMYRIGDQWDKQHDMGHMMR
       CVGNGRGEWTCIAYSQLRDQCIVDDI
```

WO 99/31233 PCT/GB98/03766

```
N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y
Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
R P V S I P P R N L G Y
```

or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

10

A still further aspect of the invention provides a method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

15

20

25

30

35

```
NLVATCLPVRASLPHRLN
MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q
INQQWERT
           YLGNALVCTCYGGSRGFNC
PEAEETCFDKYTGNTYRVGDTYERPKDSMI
    TCIGAGRGRISCTIANRCHEGG
 D T W R R P H E T G G Y M L E C V C I A E K C F D H A A G T S Y V V G E
                                   GE
                           LGNGK
    E K C F D H A A G T S
T C L G E G S G R I T
                           T W
                              E K P
                    CTSRNRCN
                                D
  С
                                 Q D
  G D T W S K K D N R G N L L Q C I C T
                                    G
                              GNGR
           TSSGSGPFTDVRAAVY
ERHTSVQT
  PPYGHCVTDSGVVYSVGMQWLKT
                                    Q
MLCTCLGNGVSCQETAVTQTYGGNSN
                          Q D Q K
F L Y N
  PFTYNDRTDST
                   T S N Y
                        Ε
       RGGNSNGAL
                    С
                      Н
                       F
                         Ρ
    0
     Т
                              NNH
              CGT
                   Т
                       YDADQ
                                    С
   G
    RR
       DNMKW
                    QN
                              KF
                                 G
                                   F
       TTNEGVMYRIGDQWDK
    I C
                              QН
                                 DMGHMMR
CTCVGNGRGEWTC
                   IAYSQLRDQ
                                CIVDD
NVNDTFHKRHEEGHMLNCTCFGQGRGRWKC
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G
                                       VRY
     YGRGIGEWHCQPLQTYPSSSGPVEVF
 C Y C
  ETPSQPNSHPIQWNAPQPSHISKYILRW
VSIPPRNLGY
```

or a natural variant or fragment thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

10

15

25

WO 99/31233 PCT/GB98/03766

Preferably, the reagent which can distinguish the polynucleotide encoding MSF from the polynucleotide encoding fibronectin is a suitable polynucleotide as disclosed herein. Methods of detecting specific nucleic acids in a sample are well known in the art. For example, *in situ* hybridisation methods which detect mRNA may be used, and northern blotting methods may be used. Dot blots, slot blots and Southern blots may also be used.

Thus, it can be seen that the reagents used in the above methods may be used in the manufacture of a reagent for diagnosing cancer.

It will be appreciated that the antibodies of the invention, and the polynucleotides of the invention, which can distinguish MSF and fibronectin (particularly those which recognise MSF or a nucleic acid encoding MSF, but not fibronectin, or a nucleic acid encoding fibronectin) are useful packaged into diagnostic kits containing said antibodies or polynucleotides and other reagents such as means for labelling the said antibodies or polynucleotides.

The invention also includes a number of therapeutic applications, for example chemoprevention and chemotherapy.

Chemoprevention includes the neutralisation of MSF activity and/or the suppression of inappropriate MSF expression in individuals deemed to be at risk of cancer due to inappropriate MSF production. It may also be desirable to administer inhibitors of MSF. Antibodies directed at MSF may act as inhibitors.

20

25

Chemotherapy includes the use of anti-MSF antibodies to target coupled cytotoxins to sites of inappropriate MSF production, and the use of MSF inhibitors as mentioned above.

Antibody-targeted cytotoxins are well known in the art and include antibodies linked to a directly cytotoxic moiety such as ricin or a toxic drug; and antibodies linked to an indirectly cytotoxic moiety such as an enzyme which is able to convert a non-toxic prodrug into a toxic drug. In the latter case, the prodrug as well as the antibody-linked enzyme is administered to the patient.

It is useful to measure MSF in wound fluids since this information may be relevant in terms of predicting the efficiency of the subsequent healing process, including the propensity of the scar. The amount of MSF in wound fluids may be measured using, for example, an MSF-selective antibody of the invention.

Inappropriate expression of MSF may be a feature of several pathological conditions characterised by inflammation, such as rheumatoid arthritis. The measurement of MSF in associated body fluid, such as synovial fluid, may be of clinical utility; other pathological conditions of relevance in this context include fibrotic and periodontal disease.

MSF is believed to be involved in the migration of cells, especially fibroblasts any, in particular, the migration of cells may take place within the wound.

Thus, a further aspect of the invention provides a method of modulating cell migration the method comprising administering an effective amount of

WO 99/31233 PCT/GB98/03766

a polypeptide of the invention to the site where modulation of cell migration is required.

Typically, the cell whose migration is modulated is a fibroblast. Typically, MSF stimulates the migration of cells such as fibroblasts. Preferably, the site where modulation of cell migration is required is a site within a mammalian body, such as the body of a horse, pig, cow, sheep, cat, dog and the like. Most preferably it is a site within a human body. It is preferred if the site within the body is the site of a wound.

10

A further aspect of the invention provides a method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide of the invention.

The invention also includes a method of preventing scarring by administering to the locality of the site where scarring is believed to be likely an effective amount of an MSF polypeptide as described herein or a suitable fragment or variant. Preventing or reducing scarring may be part of the wound-healing process. The MSF polypeptide as described herein or a suitable fragment or variant is believed to be useful in preventing or reducing scarring because it reduces hyaluronic acid formation.

It is preferred if the polypeptide administered is a recombinant polypeptide expressed in a eukaryotic host.

25

The MSF polypeptide may be administered to the site of cell migration or wound healing by any suitable means. Conveniently, the polypeptide is administered topically. It is particularly preferred if the polypeptide is incorporated within an applied wound dressing such as a collagen mesh.

15

WO 99/31233 PCT/GB98/03766

Dressings which are suitable for the incorporation of the polypeptide of the invention are well known in the art and many are commercially available.

Other formulations might involve the incorporation of MSF into an ointment, paste, gel, cream (or equivalent) designed for topical application.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (polypeptide of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

25

20

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient WO 99/31233 PCT/GB98/03766

in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Application of gene therapy techniques may provide a means of controlling MSF expression.

Any suitable amount of the polypeptide of the invention may be administered. By "suitable amount" we mean an amount which gives the desired biological response and that does not lead to any significantly undesirable effects such as toxicity or the like. Small quantities of MSF, for example less than 1  $\mu$ g, may be effective. It is preferred if superficial wounds, such as those to the skin, are treated by the method of the invention.

20

25

15

The invention will now be described in further detail with reference to the following Figures and Examples wherein:

Figure 1 shows the entire nucleotide sequence of the 2.1kb insert in clone  $pMSF1\alpha$  which contains the MSF cDNA. The start and stop codons are underlined.

Figure 2 shows the translation of the cDNA sequence shown in Figure 1 and the alignment of the peptide sequence with that of the gelatin-binding

WO 99/31233 PCT/GB98/03766

domain of fibronectin. The start and end of the MSF polypeptide are indicated by vertical bars and arrows.

Figure 3 shows the peptide sequence of MSF (as encoded in the pMSF1 $\alpha$  clone) according to its domains. The sequence of pMSF1 $\alpha$  is shown grouped according to its domains (cf analysis of fibronectin from Kornblihtt *et al* (1985) *EMBO J.* 4, 1755-1759). Residues are numbered and have been aligned to give optimal homology by introducing gaps (indicated by ^). Identical residues within a type of homology are indicated by a box (A), and stop codons are designated by asterisks (\*). Deleted amino acids are indicated by dashed lines (-), and the IGDS sequence is underlined.

Figure 4 shows a diagrammatic comparison of fibronectin and MSF.

15

Figure 5 shows a diagrammatic model of MSF showing the positions of the IGD-containing sequences (ie. IGDT, IGDS and IGDQ) within the domains.

20 Example 1: Cloning and sequence analysis of pMSF1 $\alpha$ , a clone encoding MSF

A cDNA library was constructed using mRNA extracted from a human foetal fibroblast cell line, MRC5-SV2, in the vector  $\lambda$ ZapII.

25

A primer based on peptide sequence from the gelatin-binding domain (GBD) of fibronectin was used together with a vector primer in the polymerase chain reaction (PCR) to amplify a fragment of 1.2kb. Sequence analysis showed a strong homology to GBD for most of the

10

15

20

25

WO 99/31233 PCT/GB98/03766

fragment. Clear differences included an internal deletion of 45bp, and a 3' unique sequence of 175bp.

The 3' unique sequence was used as a probe for screening the library, using the digoxigenin-labelled system. Positive plaques were picked for further analysis by secondary and tertiary screening, followed by *in vivo* excision of the pBluescript™ phagemid containing the cloned insert.

A plasmid containing an insert of 2.1kb, named pMSF1 $\alpha$ , was sequenced by the Sanger-dideoxy method, using a progressive priming approach, and the sequence was assembled into a single contain using the Fragment Assembly System of the Daresbury/Sequet series of programs.

The entire nucleotide sequence of the 2.1kb fragment is shown in Figure 1.

Translation of this sequence and alignment of its peptide sequence with that of the gelatin-binding domain of fibronectin was achieved using the Fasta program (Daresbury/Sequet), and is shown in Figure 2.

Figure 3 shows the peptide sequence of pMSF- $1\alpha$  grouped according to its domains.

Other cDNA clones encoding MSF may be readily obtained and sequenced using methods well known in the art and probe derived from the Figure 1 sequence, in particular probes which distinguish MSF from fibronectin.

15

20

# Example 2: Demonstration of the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast tissue

In situ hybridisation using a riboprobe based on the unique coding region for the unique C-terminus of MSF demonstrates the presence of MSF-secreting fibroblasts in sections of breast cancer, but not normal breast tissue.

Suitable riboprobes contain the entire unique nucleotide sequence of MSF- $1\alpha$  (position 1953-2147), and may include up to 10 bases upstream and contained within the fibronectin sequence (position 1943-2152). This ensures high specificity towards MSF- $1\alpha$ , whilst allowing the use of a probe of longer length. A digoxigenin-labelled riboprobe containing a major portion of the unique sequence (position 1974-2147) is used. This region was selected on the basis of the position of convenient restriction sites.

## Example 3: Monoclonal antibodies which are specific to MSF and do not cross-react with fibronectin

Monoclonal antibodies are raised using any of the currently available standard procedures. The immunogen is a synthetic peptide based on the 10 amino acid unique tail of MSF or is based on the peptide sequences:

25 ISKYILRWRP<u>VSIPPRNLGY</u>; or QQWERTYLGN<u>A</u>LVCTCYGGSR; or PCVLPFTYN<u>DRTD</u>STTSNYEQDQ; or TDHTVLVQT<u>R</u>GGNSNGALCH; or VGNGRGEWTCIAYSQLRDQCI

15

20

#### Example 4: Genomic PCR and FISH studies

Objective: To obtain information regarding the sequence of the genomic MSF gene regarding (i) its relationship to fibronectin, and (ii) chromosomal location.

Background: The 5' upstream untranslated sequence of the cloned MSF cDNA is identical to that of fibronectin, thereby strongly suggesting its close relationship to the fibronectin gene (note: such upstream untranslated regions are virtually never identical between two genes as there is no selective pressure. This inference is in apparent conflict with the "uniqueness" of the 3' end of the MSF cDNA which codes for a 10 amino acid polypeptide and also contains a contiguous untranslated region containing several stop codons).

Methods and Results: Two PCR reactions were established: one at the extreme 5' untranslated region of fibronectin (FN)/MSF and the other at the extreme 3' region of MSF which encompassed the 175bp unique sequence. Reactions were carried out using DNA purified using the *Qiagen* Blood kit. Sequence analysis of the resulting amplicon revealed that the 175bp "unique" sequence was contiguous with the fibronectin sequence.

Experiments were then carried out in order to obtain initial data regarding the genomic location of the 3' unique sequence. This was accomplished by selecting clones from the human PAC library (obtained from HGMP) using the above 2 PCR approach. Secondary and tertiary screening lead

to the identification of on which produced products from both PCR reactions. This clone was approximately 70-110 kb in size.

The isolated clone was next subjected to restriction digestion (BamHI and KpnI) and the fragments subcloned into pBluescript and analysed using our 2 PCR approach. Two positive clones were identified: clone B3(2) is 20 kb and can generate both the 5' and 3' fragments, thereby indicating that it contains the entire MSF genomic sequence. The other clone, K5(5) is 7 kb and only contains the 3' unique sequence.

10

We have used both clones for FISH analysis of the human genome. Our data unambiguously indicate that MSF maps to chromosome 2 region q35. Note: this is within the fibronectin gene, which is located on chromosome 2q34-36.

15

Conclusions: The FISH analysis clearly indicates that the gene coding for the MSF "unique" sequence is contained within the fibronectin gene. These results indicate that MSF is a novel "mini" splice variant of fibronectin. The genomic fibronectin gene is very large indeed and has still not been fully sequenced. To our knowledge, this is the first report of the unique sequence. The absence of the unique sequence in all previously identified isoforms of fibronectin (which are all in excess of 220 kDa compared to 70 kDa for MSF) indicates that it is spliced out of these molecules.

25

20

This information is of relevance for several reasons. Firstly, all previously described splice variants of fibronectin have molecular masses in the region of 225 kDa compared with only 70 kDa of MSF. This small size is totally unexpected and prompts us to refer to MSF as a novel

5

15

20

25

WO 99/31233 PCT/GB98/03766

"mini" splice variant of fibronectin. Secondly, all known splice variants of fibronectin involve the inclusion/deletion of entire type III repeats or variable regions of the IIICS region (all of which occur at a considerable distance downstream of the termination of MSF, which does not contain any known splice site). Finally, as the unique 3'-sequence of MSF was not hitherto identified, it was not possible to predict that MSF was indeed a splice variant of fibronectin until the above data was obtained from genomic DNA.

### 10 Example 5: Recombinant MSF expression

Objective: To express recombinant human MSF (rhMSF) in 3T3 cells.

3T3 cells were transfected using Methods and Results: Lipfectamine/Plus system (Gibco), according to the manufacturer's The plasmid used was pcDNA3.1/hisB/lacZ. instructions. sequence contained a sequence encoding a his tail fused to the human MSF cDNA sequence so that a fusion protein with a his tail is expressed. This facilitates purification of the expressed protein. Transfectants were isolated by their selective growth in medium containing 418. One liter of conditioned medium produced by the transfected cells was collected and the fraction containing all the migration stimulating activity obtained by doing a 0-20% ammonium sulphate precipitation. The pellet was resuspended in buffer and the his-tagged rhMSF purified by passage through a ProBond column (Invitrogen) column, all done in accordance with manufacturer's instructions. Approximately 250 µg of rhMSF were collected from the starting material. The purified protein resulted in a single band of approximately 70 kDa in SDS PAGE. This protein stimulated the migration of target adult fibroblasts and was active at

15

20

concentrations between 1 pg/ml to 10 ng/ml (ie in precise agreement with previously published data regarding the dose-response of MSF purified from fetal fibroblast conditioned medium).

### 5 Example 6: Anti-MSF antibody production

Objective: To generate polyclonal antibodies to MSF.

Methods: Rabbits were immunised with a 15-mer synthetic peptide based on the C-terminus of MSF: note, this contains the entire 10 amino acid unique sequence and the contiguous 5 amino sequence of fibronectin. The synthetic peptide was coupled to keyhole limpet haemocyanin (KLH) carrier and used to immunise two rabbits with the following protocol: first injection of 10 mg and second injection of 5 mg three weeks later. Serum was collected six weeks after the first injection and purified IgG shown to recognise the synthetic peptide in both dot and Western blots.

Results: We have used the antibody for both Western blots and immunohistochemistry. The former application has (i) confirmed that rhMSF is recognised by the antibody, and (ii) demonstrated that fetal, but not adult, fibroblasts produce a 70 kDa molecule which is recognised by the antibody and expresses migration stimulating activity when eluted from the PAGE gels.

Polyclonal antibodies were generated against a synthetic peptide incorporating the 10 amino acid "unique" MSF C-terminal sequence. This antibody recognises the unique synthetic peptide (down to 5 ng) and MSF (down to 10 ng) in dot blots; it does not recognise fibronectin or BSA at concentrations up to 4 μg. This antibody has been used to

WO 99/31233 PCT/GB98/03766

investigate the tissue distribution of MSF; these experiments show that MSF is present in the stromal compartment of fetal skin and is not detectable in adult skin.

35

#### **CLAIMS**

1. A recombinant polynucleotide encoding a polypeptide comprising the amino acid sequence

```
NLVATCLPVRASLPHRLN
5
   MLRGPGPGLLLLAVQC
                                   GTAVPSTGASKSK
                                 L
                                 Q S K P G C Y D N G K H Y
                Q P Q S P V A \overline{V} S
         O O M V
                  YLGNALVC
                                 Т
                                   CYGGSRGFNCESK
           WERT
                         GNTYRVGDTYERP
     EAEETCFDKYT
             I G A G R G R I S C T I A N R C H E G G
10
       CTC
                                     CLGNGKGEWTCK
         WRRPHETGGYMLECV
     I A E K C F D H A A G T S Y V V G E T W E K P Y D C T C L G E G S G R I T C T S R N R C N D Q D
                                                  QG
                                                  Т
    RIGDTWSKKDNRGNLLQCICTGNGR
                    S S
                        G S G
                             Ρ
                               F
                                 Т
                                   D
                                     V
                                       R A
                                          A V
                                              Y
                                                  Ρ
15
    ERHTSVQTT
                          G
                                 S
                             V
                               Y
                      D
                        S
                            V
                                   V
                                     G
                                          M
                                            L
                                              K
                                                T
                                                  Q
     P P P
           YGHC
                  V
                    T
                                       M Q
               \mathsf{G}\ \mathsf{N}\ \mathsf{G}\ \mathsf{V}
                             Т
                               Α
                                 V T
                                         Y
                      s c
                          QΕ
                                     Q
                                       Τ
                                          GGNS
         T
           С
             L
                      D S
                          T
                            Т
                             S N
                                 ΥE
                                     QDQK
                                            Y
                                              S
                                                F
         F
             Y
               NDRT
       Ρ
           T
              G G N S N G A L
                             С
                               H F
                                   Ρ
                                     F
                                       L
                                         YNNHNY
       V
         Q
           T
             R
           R D N M K W C G T T Q N Y D A D Q K F C T T N E G V M Y R I G D Q W D K Q H
         RRDNMKWCGTT
                                                F C
20
                                              G
       G
                               GDQWDKQHDMG
         Ι
                           I A Y S Q L R D Q
H M L N C T C F G
       CVGNGRGEWTC
                                              IVD
                                            С
    NVNDTFHKRHEEGHM
                                            Q
                                              GRG
     PVDQCQDSET
                        G
                          TF
                              Y
                               QIG
                                     DSWE
                                            K
           YGRGIGEWHC
                             Q
                                 L
                                     T
                                       Υ
                                         Р
                                            S
                                                G
25
         C
                               Ρ
                                   Q
                                           S
                                              S
                  N S H P I Q W N A P Q P S H I S K Y I L R W
           Ρ
             S Q P
    RPVSIPPRNLGY
```

or variants or fragments or derivatives or fusions thereof or fusions of said variants or fragments or derivatives.

- 2. A polynucleotide according to Claim 1, encoding a polypeptide comprising the amino acid sequence shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660, or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.
- 3. A polynucleotide according to Claim 1 or 2, which contains no introns.
- 40 4. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1.

TETP

RPVSIPPRNLGY

5

- 5. A polynucleotide according to any one of the preceding claims, comprising the polynucleotide whose sequence is shown in Figure 1 between positions 57 and 1982.
- 6. A polynucleotide according to any one of the preceding claims, encoding a polypeptide which has migration stimulating factor activity.
- 7. A replicable vector comprising a polynucleotide as defined in any one of Claims 1 to 6.
  - 8. A host cell comprising a recombinant polynucleotide or a replicable vector as defined in any one of Claims 1 to 7.
- 15 9. A method of making a polypeptide having the amino acid sequence

```
LPVRASLPHRLN
    NLVATC
                         L A V
                               Q
                                 C L G T A V P S T G A S K S K
            P
               PGLL
                       L
    MLR
             G
             \verb|QMVQPQSPVAVSQSKPGCYDNGKHY| 
         Q
                   YLGNALVCTCYGGSRGFNCESK
           WERT
        Q
         Q
    PEĀĒETCFDKYTGNTYRVGDTYERPK
20
            CIGAGRGRISCTIANRCHEGG
    GDTWRRPHETGGYMLECVC
                                              G
                                                  GE
                                        LGN
                                         T W
      IAEKCFDHAAGT
                             S
                               ΥV
                                   V
                                     G
                                       E
                                            Ε
                                              K
                                                Ρ
                                                  Y
                                                      G
             LGEGSGRI
                             Т
                               C T S
                                         R
                                          C N
                                              D
                                                  D
                                     R N
                                                Q
    VDCTC
                           G
                                 L
                                         С
                                           \mathbf{T}
                                            G
                                              Ν
                                                G
                                                  R
                                                    G
25
            TWSKKDNR
                             Ν
                               L
                                   Q
                                     С
                                       I
    RIGD
                                     D V
                     S S G S
                             G P
                                 F
                                   Т
                                         R A A
                                              V
                                                Y
                   T
            S
              V
                Q
                  T
              \bar{\mathsf{G}} \bar{\mathsf{H}} \bar{\mathsf{C}} \bar{\mathsf{V}} \bar{\mathsf{T}} \bar{\mathsf{D}} \bar{\mathsf{S}} \bar{\mathsf{G}}
                             V
                               V
                                 YSVGMQWLK
          P
            Y
        Ρ
              L G N G V S C Q E T A V T Q T Y G G
Y N D R T D S T T S N Y E Q D Q K Y
                                           Ÿ
                                             GGNSN
        С
          T
            С
                                                 S
                                                  F
    V L P F
    V L V Q T R G G N S N G A L C H F
                                     Ρ
                                         LY
                                             N
                                       E
30
                               Q N
I G
    SEGRRDNMKWC
                         G
                           Т
                             Т
                                   Y
                                     D A
                                         D
                                           Q
                                             K
                                              F
                                                   F
                           Y
                                       WDK
                                                 D
                                                  M G
              тт
                  ΝE
                      G
                       V
                         Μ
                             R
                                 G
                                   D
                                     Q
                                             QH
          ΙC
                             I A Y S
                           С
                                       LRDQ
                                              С
                                                 Ι
                                                   V D D
                    GEWT
                                     Q
            GNG
                  R
              FHKRHEEGHMLN
                                       T C
                                           FGQ
                                                 G R G
                                     С
          DT
            QCQDSETGT
                             F
                               YQIGDSWEKYV
                                                    Н
                                                      G
                                                        V R Y
35
        V
          D
            Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F
        Y C
```

or variants or fragments or fusions or derivatives thereof, or fusions of said variants or fragments or derivatives, the method comprising culturing a host cell as defined in Claim 8 which expresses said variant or fragment

SQPNSHPIQWNAPQPSHISKYILRW

35

or derivative or fusion and isolating said polypeptide or variant or fragment or derivative or fusion from said host cell culture.

10. A polypeptide comprising the amino acid sequence

```
NLVATCLPVRASLPHRL
5
   MLRGPGPGLLLLAV
                         Q
                            LGTAVPSTGASKSK
                         \overline{\mathtt{V}} S Q S K P G C Y D \overline{\mathtt{N}}
                                           GKHYQ
                      V A
    QAQQMV
              0
               Ρ
                 QSP
                            T C
                 LGNALVC
                               YGGSRGFN
                Y
           E R T
      0
         W
    EAEETCFDKYTGNTYRVGDTYERPKDSMI
           I G A G R G R I S C T I A N R C H E G G Q
10
   WDC
        T C
        WRRPHETGGYMLECVCLGNGKGEWT
      AEKCFDHAAGTSYVVGETWEK
                                       PΥ
                                            G
        TCLGEGSGRITCTSRNRCND
                                       Q
                            Q
T
                                           G
   RIGDTWSKKDNRGN
                         L
                           L
                              С
                                I
                                 С
                                  T
                                    G N
                                       G R
                                 R A
                                V
                                       Y
   ERHTSVQTTSSGSGPF
                              D
                                    Α
                                      V
                                           Ρ
15
                                GMQWLKT
                            s v
                                           0
           GHC
                V T D S
                      G
                       V
                         VY
     ΡP
        Ρ
         Y
                                   YGGNSN
           L G N G
                 V S
                    С
                         T A
                            V T
                                QT
                      Q
                        Ε
        T
         С
           YNDRTDS
                      Т
                        Т
                         S N
                            ΥE
                                Q D Q K
                                      Y
                                        SF
        F
         T
      Ρ
                                 LYNNHNY
        QTRGGNSNGAL
                                F
                         CHFP
     L V
              M K W C G T T Q N Y D A D Q K F G F C N E G V M Y R I G D Q W D K Q H D M G
20
        RRDNMKWCG
    E G
        I
           ТТ
         С
      CVGNGRGEWTCIAYSQLRDQ
                                      С
   NVNDTFHKRHEEGHMLNC
                                Т
                                 С
                                   F
                                    G
                                        G
                                         R G
                                   WEKY
                             I G D S
                                         V H G
   D P V D Q C Q D S E
                    G
                      Τ
                        F
                         Y
                           Q
                IGEWHC
                                      S S
                                         G
                         Q
                                T Y P S
         YGRG
                           PLQ
25
        С
           SQPNSHPIQWNAPQPSHISKYIL
        T
      E
          Ρ
   RPVSIPPRNL
                   G Y
```

or variants or fragments or fusions or derivatives thereof or fusions of said variants or fragments or derivatives.

- 11. A polypeptide according to Claim 10, comprising the amino acid sequence shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660, or variants or fragments or fusions thereof or fusions of said variants or fragments.
- 12. A polypeptide obtainable by the method of Claim 9.
- 13. A polypeptide according to any one of Claims 10 to 12, which has migration stimulating factor activity.

14. An antibody reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
    MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
                      QSPVAVS
                                                Y
    RQAQQMVQP
                                    QSKPGC
                    YLGNALVCTCYGGSRGF
            WERT
    PEĀĒETCFDKYTGNTYRVGDTYERPKD
    W D C T C I G A G R G R I S C T I A N R C H E G G Q S Y G D T W R R P H E T G G Y M L E C V C L G N G K G E W T P I A E K C F D H A A G T S Y V V G E T W E K P Y Q G W
10
    V D C T C L G E G S G R I T C T S R N R C N D Q D T R T
    R I G D T W S K K D N R G N L L Q C I C T G N G R G E W E R H T S V Q T T S S G S G P F T D V R A A V Y Q P Q P
                    VTDSGV
                                VYSVGMQWLK
              GHC
            Y
            CLGNGVSCQETAV
                                            YGGNS
15
        СТ
                                      T Q T
    V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C T
V L V Q T R G G N S N G A L C H F P F L Y N N H N Y T
    S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
    HEEICTTNEGVMYRIGDQWDKQHDMGHMMR
20
        CVGNGRGEWTC
                              IAYS
                                      Q L R D Q C
C T C F G Q
                                                    V
          DTFHKRHEEGHML
                                    N
                                                  G
                  DSET
                          G T F
                                  QIGDSWEKYV
                                Y
        V D Q
              С
                Q
    QCYC
            YGRGIGEWHCQPLQTYPSSSG
                Q P N S H P I Q W N A P Q P S H I S K Y I L R W
    ITETPS
25
    RPVSIPPRNLGY
```

or natural variants thereof but not reactive towards fibronectin.

- 15. An antibody reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof but not reactive towards fibronectin.
  - 16. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is

```
35
       NLVATCLPVRASLPHRLN
       MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
       R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N
I N Q Q W E R T Y L G N A L V C T C Y G G S R G F
                                           TGNTYRV
                 ĒET
                           С
                              FDKY
                                                                  G D T
                                                                           Y
                                                                               E R
                  \texttt{T} \ \texttt{C} \ \texttt{I} \ \texttt{G} \ \texttt{A} \ \texttt{G} \ \texttt{R} \ \texttt{G} \ \texttt{R} \ \texttt{I} \ \texttt{S} \ \texttt{C} \ \texttt{T} \ \texttt{I} \ \texttt{A} \ \texttt{N} \ \texttt{R} \ \texttt{C} \ \texttt{H} \ \texttt{E} 
                                                                                  GG
                 WRRPHETGGYMLE
                                                           С
                                                               VCLGNGK
          IAEKCFDHAAGTSYVVGETWEKPYQGWMM
           D C T C L G E G S G R I T C T S R N R C N D Q D T
              \texttt{G} \ \texttt{D} \ \texttt{T} \ \texttt{W} \ \texttt{S} \ \texttt{K} \ \texttt{K} \ \texttt{D} \ \texttt{N} \ \texttt{R} \ \texttt{G} \ \texttt{N} \ \texttt{L}
                                                        L
                                                           Q
                                                               С
                                                                  ΙC
                                                                        T
                                                                            G N
45
                                 T S S G S G P V T D S G V V
                                                        F
                                                                  V
           R H
                    s v
                           Q
                              T
                                                            Т
                                                               D
                                                                     R
                                                                        Α
                                                                               V
                                                                                  Y
                 T
                                                                           Α
                    YGHC
                                                        Y S
          PPP
                                                               V
                                                                  G M Q W L
              СТ
                    CLGNGVSCQETAVTQT
                                                                        YGGNS
        V L P F T Y N D R T D S T T S N Y E Q D Q K Y S F C V L V Q T R G G N S N G A L C H F P F L Y N N H N Y
50
        S E G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A
H E E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
```

20

```
C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

or natural variants thereof but which epitope is not present in fibronectin.

- 17. An antibody reactive towards an epitope present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660 or natural variants thereof but which epitope is not present in fibronectin.
- An antibody according to any one of Claims 14 to 17, reactive 15 18. comprising peptides of towards molecule any one the ISKYILRWRPVSIPPRNLGY or QQWERTYLGNALVCTCYGGSR or **EPCVLPFTYNDRTDSTTSNYEQDQ** CTDHTVLVQTRGGNSor NGALCH or VGNGRGEWTCIAYSQLRDQCI.

19. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
                 \texttt{G} \; \texttt{L} \; \texttt{L} \; \texttt{L} \; \texttt{L} \; \texttt{A} \; \texttt{V}
                                  LGTAVPSTGASKSK
    MLRGPG
               Ρ
                               Q
                                 С
                      \verb|QSPVAVSQSKPGCYDN| 
25
    R Q A
         Q
           Q M V
                 Q
                   Ρ
                   YLGNALVCT
                                    С
                                       YGGSRGF
                                                    N
          Q
           WERT
      EAEETCFDKYTGNTYRVGDT
                                             Y
                                              E
                                                  Ρ
                                                    K
             IGAGRGRI
                             S C
                                 Т
                                   I
                                     ANRC
                                             Н
      DCTC
                 H E T G
                         G
                           Y
                             Μ
                               L
                                 Ε
                                   С
                                     V
                                       С
                                         L G
                                             N
                                               G
                                                 K
                                                  G
                                                     E
      DT
         W
           RRP
                               Y
                                 V
                                   V
                                             Ε
30
          Ε
            K C
               F
                 D
                   HAAG
                           T
                             S
                                     G
                                       Ε
                                         T W
                               С
                   G S G R I
                             T
                                 TSRN
                                         R
                                           C N
                                               D
                                                 O D
            С
                G
                 E
        С
          T
              L
          D
            T
              W
               S
                   KDNRGN
                               L
                                 L
                                   Q
                                     С
                                       Ι
                                         С
                                           T
        G
                 K
      I
                                 F
                     SSGSG
                               Ρ
                                   T
                                     D
                                       VRAA
                                                 Y
            S
              V Q
                   Т
                                               V
      R H
          Т
                 T
                 CVTDSGVVYSV
                                       GMQ
                                             WL
                                                 K
                                                   \mathbf{T}
              G H
          Ρ
            Y
             LGNGVSC
35
        С
          T
            С
                           Q E T A V
                                     Т
                                       Q
                                         T
                                             G
                                               G
          F
                       D S
                           Т
                             T
                               S
                                 N
                                   Y
                                     E
                                       Q
                                         D
                                           0
                                             K
        Ρ
            Т
              Y
                N
                 D R
                     T
                             L
                           Α
                               С
                                 Η
                                   F
                                     P
                                       F
                                         L
                                             N
                                               N
                                                 Н
                                                   Ν
            T
              R
                G
                  G
                   N
                     S
                       N
                         G
        V
          Q
                           T
                             Т
                               Q
                                   Y D A D
                                             K
                                               F
                       С
                         G
                                 Ν
                                           Q
                                                 G
          R
            R
              D
               N
                 М
                   K W
                                   D Q
                                       WD
              Т
                T
                  N
                   E G
                       V M Y R I G
                                           K
                                             Q
                                               H D M G
            С
        Ε
          Ι
                                 Y S Q
40
          VGNG
                 R G
                     EWTC
                             I
                               Α
                                       L R D
                                             Q
                                               С
                                                 I
                                                   V D
      Т
      V N D T F H K R H E E G H M L N C
                                               QGRGR
                                       T
                                         C F
                                             G
      P V D Q C Q
                  DSETGT
                             F
                               Y
                                 Q
                                   Ι
                                     G
                                       D
                                         S W
                                             E
                                               K
                                                 Y
                                                   V
                                                       G
            Y
                    I G E
                         WHC
                               QP
                                   L Q
                                       T
                                         Y
                                           Ρ
                                             S
                                               S S
                                                   G
                                                     Ρ
                                                       V
                                                         Ε
      С
        Y C
              G R
                  G
                           IQWNAP
                                       OPSHISKYIL
          T
            P
              S Q P
                    Ν
                      S H
                         Ρ
      T
        Ε
45
        V
          S
            IPPRN
                     L
```

or natural variants thereof.

- 20. An antibody reactive towards fibronectin but not reactive towards the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1α between positions 19 and 660 or natural variants thereof.
  - 21. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
10
                 LLLAVQCLGTAVPSTGASKSK
   MLRGPGP
              G L
                             SKPGCYDNGKHYQ
                 QSPVAVS
                            0
     QAQQMV
              QP
                             CYGGSRGFN
               YLGNALVC
                            T
              T
         W
           ΕR
                    TGNTYRVGDTY
                                     ERPK
              FDKY
            С
         Ε
           T
        E
                       SCTIANRC
            GAGRGRI
                                   ΗE
                                       G
15
        Т
         С
           Ι
      С
        W R R P H E T G G Y M L
                          E C
                                       K
                                        G
                              V
                               \mathsf{C}
                                 L
                                  G
      T
           CFDHAAGT
                               E
                                 T
                                        Y
                       S
                         Y
                           V
                            V
                              G
                                  W
                                    EKP
    IAEK
                                     DQDT
   V D C T C L G E G S G R I
                       Т
                         С
                          T S
                             R
                               N
                                 R
                                  C N
                          L
                               I C
                                  Т
                                    G N G
                    R G N L
                            Q
                              С
      GDTWSKKD
                   N
   R
                   SGSGPFT
                             DVRAAV
20
                Т
                 S
          S
           V
             Q
              T
   E
     RHT
                                  QWLKT
                   D S G V V Y S
              С
                V
                 Т
                              V G M
          Y
           G H
      Ρ
        P
                      Q E T A V
                              TQTYGGNS
                                          N
             G N G V S C
      С
        Т
          С
           L
                       TSNYEQDQKYS
                                        F
                                          С
           YNDRTDST
          T
      ₽
        F
           RGGNSNGALCHFPFLY
                                    N
                                     N H
      V
          Т
   V L
        Q
                              D
                                 DQK
                                     F
                                       G
                                         F
                                          С
   SEGRRDNMKWCGTT
                         QN
                            Υ
                               Α
25
         CTTNEGVMY
                        R
                         Ι
                           G
                            D
                              Q
                               WD
                                  K
                                    Q
                                     H
                                       D
                                         Μ
                                          G
   H E E I
                                     С
                                         V
                                  D
                                    Q
                                       Ι
                                          D
      CVGNGRGEW
                    T
                      С
                        Ι
                         Α
                           Y
                            S
                              Q
                               L
                                 Ŗ
                           L N C
                               T
                                 С
                                  F
                                    G
                                     QGR
                    E G
                        Н
                   Ε
                         М
     VNDTFHKRH
                        F
                         Y
                           QIGDSWE
                                       Y V
                                          Н
                    G
                      Т
                 E T
             Q D
                S
       V D
          0 C
                   EWHCQPLQTYPSSSGPVEVF
             R G
                ΙG
30
       Y
          Y
           G
                      IQWNAPQPSHISKYILRW
          PSQPNSHP
        T
       Ε
       V S I P P R N L
```

or natural variants thereof.

35

22. An antibody reactive towards an epitope present in fibronectin but not present in the polypeptide whose amino acid sequence is shown in Figure 2 labelled pMSF1 $\alpha$  between positions 19 and 660 or natural variants thereof.

40

23. An antibody according to any one of Claims 19 to 22 reactive towards a molecule comprising any one of the peptides

QQWERTYLGNVLVCTCYGGSR or EPCVLPFTYNGRTFYSCTTEG-RQDGHLWCSTTSNYEQDQ or CTDHTVLVQTQGGNSNGALCH or VGNGRGEWTCYAYSQLRDQCI or ISKYILRWRPKNSVGRWKEA or peptides derived from position 648 in fibronectin as shown in Figure 2.

- 24. An antibody according to any one of Claims 14 to 24 which is a monoclonal antibody.
- 25. A method of making an antibody which is reactive towards the polypeptide whose amino acid sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAV
                            Q
                              CLGTAVPSTGASKSK
    RQAQQMVQPQSP
                              SQSKPGC
                                           Y D
                                              N
                                                G
                                                  K
                         VAV
                              СТ
                                  С
                                            G
                                              F
                                                Ν
    INQQWERT
                  Y
                   L
                     G N A
                          L
                            V
                                   YGGS
                                          R
                            Τ
                              Y
                                  V
                                    GDT
                                         Y
                                           E
                                            R P
                                                K
15
              C F
                     ΥT
                          N
                                R
                  DΚ
                         G
     E A E
          E T
                     G R I
                          S
                            С
                              Т
                                IANRCHE
                                            G
           С
              G A
                  G R
         T
            Ι
     D
       С
                         YMLE
                                С
                                  V
                                    С
                                     LGNGK
                                              G
                                                E
       Т
         W
          RRPHET
                     GG
     D
                                  GETWEKP
                                              Y
     IAEKCFDHAAGTSYV
                                V
                                                Q
          CLGEGSGRITCTSRNRCND
                                            Q D
    V D
       С
         Τ
       G D T W S K K D N R G N L L
                                         G
                                  С
                                    I C
                                       T
                                           N
                                              R
20
                                Q
     Ι
                                             Y
                     SG
                         S
                           G
                            Ρ
                              F
                                  D
                                    V
                                      R
                                         Α
                                           V
     RHTSVOTT
                   S
                            V
                                S
              H C
                  V
                    T
                     D
                       S
                         G
                           V
                              Y
                                  V
                                    G M Q
                                         WLK
       Ρ
         PΥ
            G
                     S C
                           Ε
                            T A
                                V
                                  Т
                                       Y
                                         GGNSN
         Т
                  G V
                         Q
                                    Q
                                     T
       С
              G N
              NDRTDS
                         Τ
                           T
                            SNYE
                                    QDQKYSF
            Y
       ₽
         F
           Т
25
       V
              {\tt G} \ {\tt G} \ {\tt N} \ {\tt S} \ {\tt N} \ {\tt G} \ {\tt A} \ {\tt L}
                            CHFP
                                    F L
                                       YNNHNY
         Q
           T
            R
    V
     Ĺ
         R R D N M K W C G T T Q N Y D A D Q K F G F C P I C T T N E G V M Y R I G D Q W D K Q H D M G H
                                              F C P
    SEG
    H E E
       CVGNGRGEWTCIAYSQLRDQC
                                             I
                                               V
    NVNDTFHKRHEEGHMLNC
                                     С
                                       F
                                               R
                                                G
                                    T
                                         G
                                           Q
                                             Y
                                                    V
30
                     ΤG
                         TF
                             Y
                                Ι
                                  G
                                    D
                                     S
                                       W
                                         E
                                           K
                                               V
                                                Н
    DPVDQCQDSE
                              0
           YGRG
                  Ι
                    G
                     EWHC
                             Q
                              Ρ
                                L Q
                                    T
                                     Y
                                       P S
                                           S
                                            S
                                               G
                                                Ρ
                                                  V
                                                    Ε
     С
       Y C
                     HPIQWNAPQPSHISKYILRW
           PSQP
                    S
       E
         T
                  N
       VSIPPRN
                    L
```

- or a natural variant thereof and which is not reactive with fibronectin, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes MSF from fibronectin and selecting an antibody which binds MSF but does not substantially bind fibronectin.
- 40 26. A method of making an antibody which is reactive towards fibronectin and which is not reactive towards the polypeptide whose amino acid sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAV Q CLGTAVPSTGASKSK RQAQQMVQPQSPVAV Y D SQSKPGC NGK WERTYLGNA L V С T C Y G G S QQ T V Y PEAEETCFDKY T G N Y R G D T Ε R Ρ С Τ IANRCHE G RGRI S G D C GAG T С I H E T G G Y М L Ε С VCLGN G K Т R R Р SYVVGETWEKPY D H A A G T Α Ε K С F EGSGRITCTSRNRCNDQDT G С T C  $_{
m L}$ ICTGNGRGEW KKDNRGNL L Q С 10 G DT W S SGSGPF T D V R A A V Y Q RH T S V Q T T S GHCVTDSGVVYSVGMQWLKT P Y CTCLGNGVSCQETAV T Q T Y GGNS T T A L PFTYNDRTDS Q Y S F S N Y Ε Q D K С Ÿ N N H N G Н F ₽ F L VOT RGGN S Ν 15 Q N Y I G D T Т DADQK K W CG F GRRD N Μ NEGVMYR Q WDK Q H D M G H M M RС Т T GNGRGEWT IVDD С IAYS Q L R D Q C V V N D T F H K R H E E G H M L N C T C F G Q G R G R W K QDSETGTFYQIGDSWEKYVHG V 20 PVDQC YCYGRGIGEWHCQPLQTYPSSSGPVE TETPSQPNSHPIQWNAPQPSHISKYILRW RPVSIPPRNLGY

- or a natural variant thereof, the method comprising the steps of, where appropriate, immunising an animal with a peptide which distinguishes fibronectin from MSF and selecting an antibody which binds fibronectin but does not substantially bind MSF.
- 30 27. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards the polypeptide whose sequence is
- MLRGPGPGLLLLAVQCLGTAVPSTGASKSK QSPVAV SQSKPGC 35 RQAQQMV QΡ R G QWERTYLGNA L V С T G N T R V ΥE ΚY T G Y G D Т R ΕE T С F D RGRI S С T RCHEG A N G Α G I CLGNGKGE HETGGYMLEC V R R Ρ F D H A A G T S Y Y V V GE TWEKP 40 С Ε K G E G S G R I T C T S R N R C N D S K K D N R G N L L Q C I C T G N DT L Т С G D T W S K K D N R G N L R G R H T S V Q T T S S G S G P F T D VRAA V YGHC V Т D S G V V Y S V G M QW L K P P Р Α V T Y С E T Т G G N S G V S Q Q 45 С Т C  $\mathbf{L}$ G N Т D S  $\bar{\mathrm{T}}$ Т SNYE Q D QK Y S F F T Y Ρ N D R N S N V L V Q T R G G G A L C H F P F L YNNHNY QNYDADQKFGFCP RRDNMKW C G TT G CTTNEGVMYRĪGDQWDKQHDMG Y S Q L R 50 С GNGRGEWT С ΙA D Q С V N V DT HKRHEE G н м L N С T С F G Q G R G N F IGDSWEKY C Q D S E Τ G T F Y Q VHGV RGIGEWHCQPLQTYPSSSGPVEVF

NLVATCLPVRASLPHRLN

40

ITETPSQPNSHPIQWNAPQPSHISKYILRWRPVSIPPRNLGY

or natural variants thereof but not reactive towards fibronectin.

28. A molecule which is capable of, following immunisation of an animal if appropriate, giving rise to antibodies which are reactive towards fibronectin but not reactive towards the polypeptide whose sequence is

	N	L	V	Α	Т	С	L	Ρ	V	R	Α	S	L	Ρ	Η	R	L	N												
10	М	Τ.	R	G	P	G	P	G	Τ.	۲.	L	L	Α	V	0	С	L	G	T	Α	V	Ρ	S	T	G	Α	S	K	S	K
10	D	7	2	0	ñ	м	77	Õ	Þ	<u>_</u>	S	P	V	Α	v	S	0	S	K	Ρ	G	С	Y	D	N	G	K	Η	Y	Q
	Τ.	N.	7	×	ᅜ	E.	D	Ψ.	v	¥ T.	c	N	Δ	Τ.	v	Ċ	Ť	Ċ	Y	G	G	S	R	G	F	N	С	Ε	S	K
	ъ т	EN EN	ν	Ω.	VV	L.	~	F.	Ļ	v	v	Δ.	G	N	т	Y	R	v	Ġ	n	T	Ÿ	E	R	P	K	D	S	M	Ι
	17	Ē	A	E.	-	1	0	7	0	D.	÷	Ð	7	2	ċ	Ť	T	Δ	N	B	ċ	Н	E	G	G	0	s	Y	ĸ	T
1.5	W	ט		T	, L	Ţ	G	A	G	71	9	~	v	M	T.	T.	Ċ	77	7	T.	G	N	G	ĸ	G	E.	W	T	Ĉ	ĸ
15	G	ע	T	W	K	K	5	п	Ľ	7	7	G	LL:	L.1	7	7.7	17	Č	-	T	TAT	r	v.	D	v	0	Ġ	W	М	M
	Р	1	A	E	K	C	F.	ט	H	A	A	6	1	D D	7	m	۷	5	NI NI	D T	~	N	D	_	D.	Ϋ́	D	Ţ	2	V
	V	D	С	Т	С	L	G	E	G	S	G	R	1	T	Ţ	1	٥	7.	1/1	7	<u></u>	TA .	ע	Š	ם		T.	T	v	Ċ
	R	Ι	G	D	T	W	S	K	K	D	N	K	G	N	T	F	Q		7	-	Ţ	Ğ	IA	7,	7	5	-	W	11	n
	E	R	Η	T	S	V	Q	Т	T	S	S	G	S	G	ħ	ř.	T	U	٧	K	A.	A	٧	Y	Q	2	Š	P	П	-
20	Q	P	Ρ	Ρ	Y	G	Η	C	V	T	D	S	G	V	V	Y	S	V	G	M	Q	W	Т	K	T	2	G	N	L.	Q
	Μ	L	С	T	С	L	G	N	G	V	S	С	Q	Ε	Т	A	V	T	Q	T	Y	G	G	N	5	N	G	Ε	17	
	V	L	Ρ	F	T	Y	N	D	R	Т	D	S	T	T	S	N	Y	E	Q	D	Q	K	Y	S	F.	C	1	D	Н	T
	V	$\mathbf{L}$	V	Q	T	R	G	G	N	S	N	G	Α	L	С	Η	F	P	F	$\mathbf{L}$	Y	N	N	Η	N	Y	Т	D		
	S	Ε	G	R	R	D	N	М	K	W	С	G	Τ	$\mathbf{T}$	Q	N	Y	D	A	D	Q	K	F	G	F	С	P	М	A	A
25	Н	E	Ε	Ι	С	T	T	N	Ε	G	V	М	Y	R	Ι	G	D	Q	W	D	K	Q	H	D	Μ	G	Н	М	M	R
	С	T	С	V	G	N	G	R	G	Ε	W	T	С	I	Α	Y	S	Q	L	R	D	Q	С	Ι	V	D	D	Ι	T	Y
					T	F	Н	K	R	Н	Ε	E	G	Η	Μ	$\mathbf{L}$	N	С	T	С	F	G	Q	G	R	G	R	W	K	С
	D	P	v	D	ō	С	0	D	S	Ε	Т	G	T	F	Y	Q	Ι	G	D	S	W	Ε	K	Y	V	Η	G	V	R	Y
	ō	Č	Ÿ	Č	Ÿ	Ğ	Ŕ	G	Τ	G	E	W	Н	С	Q	P	L	Q	T	Y	P	S	S	S	G	P	V	Ε	V	F
30	-	т		Т	p	S	0	P	N	s	Н	P	Ι	0	W	N	Α	P	Q	P	S	Н	Ι	S	K	Y	I	L	R	M
20	R	-		_					N					~					_											
	1/	E	v	•		-	-	Τ.			_	_																		

or natural variants thereof.

35 29. A molecule according to Claim 27 which is a peptide comprising any one of the sequences

ISKYILRWRPVSIPPRNLGY; or

QQWERTYLGNALVCTCYGGSR; or

PCVLPFTYNDRTDSTTSNYEQDQ; or

TDHTVLVQTRGGNSNGALCH; or

VGNGRGEWTCIAYSQLRDQCI

which are found in MSF.

30. A molecule according to Claim 28, which is a peptide comprising any one of the sequences

QQWERTYLGNVLVCTCYGGSR or
EPCVLPFTYNGRTFYSCTTEGRQDGHLWCSTTSNYEQDQ or
CTDHTVLVQTQGGNSNGALCH or
VGNGRGEWTCYAYSQLRDQCI or
ISKYILRWRPKNSVGRWKEA or

peptides derived from position 648 onwards in fibronectin as shown in Figure 2.

31. A polynucleotide which is capable of distinguishing a polynucleotide which encodes the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                            QCLGTAVPSTGASKSK
   \texttt{M} \; \texttt{L} \; \texttt{R} \; \texttt{G} \; \texttt{P} \; \texttt{G} \; \texttt{P} \; \texttt{G} \; \texttt{L} \; \texttt{L} \; \texttt{L} \; \texttt{L} \; \texttt{A} \; \texttt{V}
15
    R Q A Q Q M V Q P Q S P V A V S Q S K P G C Y D N G K
         QWERTYLGNALVCTCYGGSRGF
    PEAEETCFDKYTGNTYRVGDT
                                         Y
                                          ERP
                                  ANRC
                                         ΗE
    WDCTCIGAGRGRISC
                              Τ
                                I
                                            G
                                              G
    GDTWRRPHETGG
                                     LGNG
                                            K
                                              G
                         Y M
                            L
                              E
                                С
                                  V
                                    С
20
                                  GETWEK
                                            Ρ
                                              Y
         E K C
              F
                D
                  HAAG
                         T S
                            Y
                              V
                                V
     ΙA
                            CTSRNRCNDQDT
                EGSGRIT
         T
           С
            L
              G
            WSKKDNRGNLL
                                  CICTGNGRGEWK
                                Q
         D
           Т
      I G
           D V R A A V
                                            Y
         T S V Q
       Н
                                            K
25
       P
         ₽
       CTCLGNGVSCQETAVT
                                    QT
                                         G
                                           G
                                            Ν
                                              F
       PFTYNDRTDST
                           T
                             S
                              Ν
                                Y
                                  Ε
                                    Q
                                     D
                                       Q
                                         K
                                           Y
                                            S
         QTRGGNSNGA
                                              N
                           L
                             C
                              H F
                                  P
                                    F
                                       Υ
                                         и и н
                                     L
    VLV
                           T
                             Q N Y
                                              F
                       G
                         T
                                  DΑ
                                     D
                                       Q
                                         K F
                                            G
    SEGRRDNMKWC
               TNEGVMYRIGDQWDK
                                         QHDMG
30
         I
               GRGEWTCIAYS
                                  QLRD
                                         QCIVDD
           G
         V
             N
       NDTFHKRHEEGHMLNCTCFGQGRG
           Q C Q D S E T G T F Y Q I G D S W E K Y V H
                                                  G
       V D
           Y G R G I G E W H C Q P L Q T Y P S S S G P
                                                  V E
    QCYC
    I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
35
    RPVSIPPRNLGY
```

or a natural variant thereof and a polynucleotide which encodes fibronectin.

40

polynucleotide which is capable of hybridising 32. polynucleotide which encodes fibronectin but not a polynucleotide which encodes the polypeptide whose sequence is

NLVATCLPVRASLPHRLN MLRGPGPGLLLLAVQCLGTAVPSTGASKSK RQAQQMVQPQSPVAVSQSKPGC Y D NQQWERTYLGNALVC Т С ΥG G S RGFN YRVGDT Υ E R P EAEETCFDKYTGNT s c T IANRCHEGG AGRGRI T C ΙG TGGY ECVCLGNGKGEWT М L 10 WRRP H E SYVVGETWEKP G T A A КC F DН EGSGRITCTSRNRCNDQDT G C L T G N G R TWSKKDNRGNLLQCICT D RHTSVQTTSSGSGPFTDVRAAVY PYGHCVTDSGVVYSVGMQWL 15 S Т CTCLGNGVSCQETA Q T D S T T S N G A L D Q K Y Y E Q YNDRT S N PFT FLYNNHN C H F Ρ R G G N L V QNYDADQK NMKWCGT T F GRR D  $\texttt{T} \;\; \texttt{N} \;\; \texttt{E} \;\; \texttt{G} \;\; \texttt{V} \;\; \texttt{M} \;\; \texttt{Y} \;\; \texttt{R} \;\; \texttt{I} \;\; \texttt{G} \;\; \texttt{D} \;\; \texttt{Q} \;\; \texttt{W} \;\; \texttt{D} \;\; \texttt{K}$ QHDMGHMMR 20 С T N G R G E W T C I A Y S Q L R D Q C I V D D F H K R H E E G H M L N C T C F G Q G R G R C Q D S E T G T F Y Q I G D S W E K Y V H G С V G V N DT V R Y GRGIGEWHCQPLQTYPSSSGPVEVF Y C Y TETPSQPNSHPIQWNAPQPSHISKYILRW 25 RPVSIPPRNLGY

or a natural variant thereof.

NLVATCLPVRASLPHRLN

A polynucleotide which is capable of hybridising 33. 30 polynucleotide which encodes the polypeptide whose sequence is

```
G P
                  GLLLLAV
                                 QCLGTAVPSTGASKSK
                      QSPVAV
                                   SQSKPGCYDNGKHY
            Q M V
                  Q P
    I N Q Q W E R T Y L G N A L V C T C Y G G S R G F N C P E A E E T C F D K Y T G N T Y R V G D T Y E R P K D
            CIGAGRGRISCTIANRCHEGG
                                       V C
G E
      DTWRRPHETGGYMLE
                                     С
                                           LGNG
              C F D H A A G T S
L G E G S G R I T
                                           Т
      I A E K C F D H A A G
                                 Y
                                   V
                                     V
                                             W
                                               Ε
                                                 K
                                                   P
                                                     Y
                                 С
                                   T
                                     SRNRCND
40
      D C
          \mathbf{T}
            С
                     KDNRGN
                                 L
                                   L
                                         I C
                                             T
                                               G N
                                     Q
                                       С
                  K
        G
              W
                S
                     TSSGSG
                                 P F
                                     T
                                       DVRAAVY
              V
                  T
          T
            S
                 Q
      R H
                               V V Y S V G M Q W L K T
              GHCVTD
                          S G
        Ρ
          Р
            Y
            C L G N G V S C Q E T A V T Q T Y G G N S N
T Y N D R T D S T T S N Y E Q D Q K Y S F C
        C T
                                             Q K
Y N
45
    V L V Q T R G G N S N G A L C H F
S E G R R D N M K W C G T T Q N Y
H E E I C T T N E G V M Y R I G D
                                       Ρ
                                           L
                                             QK
                                       D
                                         A D
                                                 F
                                                   G
                                                     F
                                   GDQWDK
                                               QHDMG
                                       Q L R D Q
             G
                   R G
                       EWTC
                               I A Y S
                 G
              N
                 HKRHEEGHMLNCTCFGQGRGRW
        N D
            T
              F
                 Q D S E T G T F Y Q I G D S W E K Y V H G
             Q C
        V D
             YGRGIGEWHCQPLQTYPSSSGP
                                                          V
                                                            E
         ETPSQPNSHPIQWNAPQPSHISKYI L
     RPVSIPPRNLGY
```

3 3 × 1

10

15

or a natural variant thereof but not to a polynucleotide which encodes fibronectin.

- 5 34. A polynucleotide according to any one of Claims 31 to 33, wherein the polynucleotide is an oligonucleotide.
  - 35. A polynucleotide according to any one of Claims 31 to 34, wherein the polynucleotide which encodes fibronectin or the polynucleotide which encodes the polypeptide as said or a natural variant thereof is a mRNA or a cDNA.
  - 36. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                                Q C L G T A V P S T G A S K S K
                      LLLAV
    MLRGPGPGL
                      Q S P V A V S Q S K P G C Y D N G K H Y
    RQAQQMV
                  QΡ
            WERTYLGNALVCTCYGGSRGFNC
    PEÃĒETCFDKYTGNTYRVGDTYERP
20
            \texttt{C} \;\; \texttt{I} \;\; \texttt{G} \;\; \texttt{A} \;\; \texttt{G} \;\; \texttt{R} \;\; \texttt{G} \;\; \texttt{R} \;\; \texttt{I} \;\; \texttt{S} \;\; \texttt{C} \;\; \texttt{T} \;\; \texttt{I} \;\; \texttt{A} \;\; \texttt{N} \;\; \texttt{R} \;\; \texttt{C} \;\; \texttt{H} \;\; \texttt{E}
        СТ
      D T W R R P H E T G G Y M L E C V C L
                                                G
                                                  K
                                                    G
                                            G N
    PIAEKCFDHAAGTSYV
                                          T
                                              E
                                                  Р
                                                    Y
                                    V
                                      GE
                                            W
                                                K
    V D C T C L G E G S G R I
                              TCT
                                    SRNR
                                            C N
                                                D
                                                  Q
                                        I C
                                            Т
                                              G N
                                                  G
                                  L
    RIGDTWSKKDNRGNL
                                    Q
                                      С
25
                              G P F
                                    TDVRAAV
                                                  Y
                          G S
                      S
                        S
      RHTSVQT
                    Т
                          SGVVYSVGMQWLKT
              GHC
                    V
                      T
                        D
            Y
        Ρ
            C L G N G V S C Q E T A V T Q T
T Y N D R T D S T T S N Y E Q D
                                            Ÿ
                                              G G N S N
        C
          Т
                                            QKY
        ΡF
                                      ΡF
                                          L
                                              N
                                                N
                                                  H N
            TRGGNSNGALCHF
    VLVQ
30
                                QN
                                    Y
                                      D A D
                                              K F
                                                  G
                                                    F
     SEGRRDNMKWCGTT
                                      QWDKQHDMGH
          ICTTNEGVMYR
                                I
                                  G
                                    D
                                    SQLRDQC
                                                  IVDD
                        W T
                            С
                              I
                                Α
                                  Y
          V
            GNG
                  RGE
          DTFHKRHEEGHMLNCTCFGQ
                                YQIGDSWEKYVHG
                                                          V R Y
          DQCQDSETGTF
35
            YGRGIGEWHCQPLQTYPSSSGPVEVF
        Y C
      TETPSQPNSHPIQWNAPQPSHISKYILRW
     RPVSIPPRNLGY
```

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

37. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
               P G L L L A V Q C L G T A V P S T G A S K S K
           P
           Q M V Q P Q S P V A V S Q S K P G C Y D N G K H Y Q W E R T Y L G N A L V C T C Y G G S R G F N C E S K
               CFDKYTGNTYRVGDTYERPKDS
     EAEET
             IGAGRGRISCTIANRCHEGG
10
                                       L G N G K
T W E K P
         WRRPHETGGYMLECVC
     I A E K C F D H A A G T S Y
D C T C L G E G S G R I T C
                                   G E
                               V V
                                Т
                                       R C N
                                                D
                                                  T
                                 S R N
                                            DQ
    V D C
    RIGDTWSKKDNRGN
                                L
                                   CIC
                                         T
                                           G N
                                              G R
                              L
                                 Q
                   T S S G S G P F
                                 T D V R A A V Y
                                                Q P
15
     RHT
           s v
               QT
                   VTDSGVV
                                YSVGMQWLKT
             G H C
           Y
       P
         P
             L G N G V S C Q E T A V T Q T Y G G N S
Y N D R T D S T T S N Y E Q D Q K Y S F
                                 VTOTYGGNSN
       С
         T
           С
       Ρ
         F
           Т
    V L V Q T R G G N S N G A L C H F P
                                     FLYNNHN
    SEGRRDNMKWCGTTQNYDADQ
HEEICTTNEGVMYRIGDQWDK
                                           K
                                                F
20
    HEEICTTNEGVMY
                                               D
                                                  G
                                           Q
                                            Η
                                               I
                                                V
         VGNGRGEWT
                          С
                            I A
                                Y
                                  S
                                   Q
                                     L R D
                                           Q
                                             С
     T C
         DTFHKRHEEGHMLN
                                     TCFGQ
                                               GRG
                                   С
        N
                                 IGDSWEKYV
               QDSETGTF
                              ΥQ
          D O C
                   IGEWHCQPLQTYPSSSGP
25
        Y
          C
           Y
             GRG
         T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W
       E
    RPVSIPPRNLGY
```

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

38. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
35
                  MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
                   RQAQQMVQPQSPVAV
                                                                                                                                           S Q S K P
                                                                                                                                                                                   G
                                                                                                                                                                                           С
                                                                                                                                                                                                    Y
                                           QWERTYLGNA
                                                                                                                           L V C
                                                                                                                                                           С
                                                                                                                                                                   Y
                                                                                                                                                                            G G S
                                                                                                                                                                                                   R G
                                                                                                                   GNTYRV
                                                                                                                                                                   GDTYERPKDSMI
                                                                                                           T
                                                   Ε
                                                          T
                                                                    С
                                                                           F
                                                                                    D K Y
                                                                    G A G R G R I S C T
                                                                                                                                                   I
                                                                                                                                                           ANRCHEG
                                                            I
 40
                                                                                           T G G Y M L E C
                                           WRR
                                                                   P
                                                                           H E
                                                                                                                                                           V
                                                                                                                                                                   C
                                                                                                                                                                            LGNGK
                                                                   F D H A A G T S Y V V G E T W E K P Y Q
                            IAEKC
                                          T C L G E G S G R I T C T S K N A C A D T W S K K D N R G N L L Q C I C T G N C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S C T S 
                                                                                                                                                                                                                    DT
                                                                                                                                                                                                            G
                   E R H T S V Q T T S S G S G P Q P P P Y G H C V T D S G V V
                                                                                                                                           F
Y
 45
                                                                                                                                                    S
                                                                                                                                                            V
                                                                                                                                                                    G M
                                                                                                                                                                                    QW
                                                                                                                                                                                                     L
                                                                                                                                                                                                             K
                                                                                                                    QETAV
                                                                                                                                                                                    Y
                                                                                    G
                                                                                             V S
                                                                                                            С
                                                                                                                                                            T
                                                                                                                                                                    Q
                                                                                                                                                                            T
                                                                                                                                                                                            G
                                                                                                                                                                                                     G N
                                                                                                                                                                                                                    S N
                                            Т
                                                    С
                                                            LGN
                                    С
                                                             YNDRTDS
                                                                                                                            T S N Y E
                                                                                                                                                                    QDQKY
                                                                                                                    Т
                                                                                                                                                                                                             S
                                                    Т
                                            F
                                                    TRGGNSNGALCHFP
                                                                                                                                                                   FLYNNHNY
                                            Q
                                    G R R D N M K W C G T T Q N Y D A D Q K F G F C P M A A E I C T T N E G V M Y R I G D Q W D K Q H D M G H M M R
  50
```

10

C T C V G N G R G E W T C I A Y S Q L R D Q C I V D D I T Y N V N D T F H K R H E E G H M L N C T C F G Q G R G R W K C D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y

or a natural variant or fragment thereof using a reagent which can distinguish said polypeptide from fibronectin.

39. A method according to any one of Claims 36 to 38, wherein the reagent which can distinguish said polypeptide from fibronectin is an antibody according to any one of Claims 14 to 18.

15 40. A method of diagnosing cancer the method comprising detecting in a sample from the person to be diagnosed a polynucleotide encoding a polypeptide whose sequence is

NLVATCLPVRASLPHRLN M L R G P G P G L L L L A V Q C L G T A V P S T G A S K S K 20 RQAQQMVQPQSPVAVSQSKPGCYDNGKHYQ INQQWERTYLGNALVCT CYGGSRGF AEETCFDKYT GNTYRV G D T GAGRGR I S С Т Α N R C Н Ε GDTWRRPHETGGYM LECV CLGN ΚG G PIAEKCFDHAAGTSYVVGETWEKP Y CLGEGSGRITCTSRNRCNDQD RIGDTWSKKDNRGNLLQCICTGNG ERHTSVQTTSSGSGPFTDVRAAVY CICTGNGRGEWKC Q P P P Y G H C V T D S G V V Y S V G M Q W L K Q 30 С TCLGNGVSC QΕ T A V T Q T Y GG Q T D R T D S T Y Y N T E Y F S Ν DQ K S TRGGNSNGAL C H F P F L Y N N Н Q G R R D N M K W C G T T Q N Y D A D Q K F E I C T T N E G V M Y R I G D Q W D K Q H SEGRRDNMKWCG F QHDMG CVGNGRGEWTCIAYSQLRDQ 35 CIVD NDTFHKRHEEGHMLNC T F G G T F QC Q D SET Y I G DSWE K QTYPS YGRGIGEWHC QCYC QΡ L SSGP ITETPSQPNSHPIQWNAPQPSHISKYILRWRPVSIPPRNLGY 40

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

. .

5

10

15

20

25

30

41. A method of determining susceptibility to cancer the method comprising detecting in a sample derived from the person to be tested the presence of a polynucleotide encoding a polypeptide whose sequence is

```
CLPVRASLPHRLN
                         QCLGTAVPSTGASKSK
       Р
          Р
            GLLLLAV
                OSPVAVSQSKPGCYD
                                            Ν
     Q Q M V
            Q P
              YLGNALVCTCYGGSRG
                                              Ν
     Q
      WERT
            F D K Y T G N T Y R V G D T Y
                                         Ε
EAEE
        T C
        IGAGRGRISCTI
                               ANRC
                                          G
     WRRPHETGGYMLE
                             С
                                         G
                                          K
                                            G
                               V
                                 С
                                   L
                                     G
                                       N
                      T
I
                               G E
                                   Т
                                          P
    EKCFDHAAG
                        S
                          Y
                           V
                             V
                                     W
                                       E
                                         K
                        T
                          C T
                             S R N
                                   R
                                     С
                                         D
                                          Q
                                            D
              G S G R
                                       Ν
     T
       С
        L G
            Ε
                      G
                          _{\rm L}
                            L
                             Q
                               С
                                 I C
                                     Т
                                       G N
                D
                    R
                        Ν
              K
                  N
     D
       T
         W
          S
            K
              T
V
            T
C
                             T D V R
                                     AAVY
         V
                S
                  S
                    G S
                        G P F
          Q
     Т
       S
                            YSVGMQWLKT
                          V
       Y
         G H
                \mathbf{T}
                  D
                    SG
                        V
   Ρ
     Ρ
         L G N G V S C Q E T A V T Q T
Y N D R T D S T T S N Y E Q D
                                     YGGNSN
         L G N G
   С
     Т
       С
                                     QΚ
       Т
 L P
     F
         RGGNSNGALCHF
                               Р
                                 F
                                   L
                                       Ν
SEGRRDNMKW
                    G T
M Y
                        Т
                          Q
                            N
                              Y
                               D
                                 Α
                                   D
                                     Q
                                       K
                                         F
                                           G
                                             F
                                              С
                  С
                                 WDK
                                         Н
                                          D
                                            Μ
                                       Q
         T T N E G
                  V
                        R
                          Ι
                            G
                              D
                               Q
   E I C
                      С
                        I A Y
                                 LRDQ
                                         С
                                           Ι
                    T
                              S
       GNGRGEW
                               Q
     V
           HKRHEEG
                        HMLN
                               С
                                 Т
                                   С
                                     F
                                       G
                                         Q
     D
         F
                               GDSWEKYVH
               S E T G T
                        F
                          ΥQ
                             I
         С
           0
             D
   V
     D
           R G I G E W H C Q P L Q T Y P S S S G P V E V F Q P N S H P I Q W N A P Q P S H I S K Y I L R W
   Y
     С
       Y
         G
     T
       Р
         S
   VSIPPRNLGY
```

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

42. A method of determining the likely outcome of a patient with cancer the method comprising detecting in a sample from the patient the presence of a polynucleotide encoding a polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
                                      Q C L G T A V P S T G A S K S K
                 GPGLLLLAV
35
                                      V S
V C
                                          Q
T
                                                        Y
                                               КР
                                                   G
                     QPQSPVA
              Q M V
                                                 GGSR
              WERTYLGNAL
                                             С
                                               Y
                                      T Y
C T
                                          R
                                            V
                                               G D T
                                                      Y
                                                        E
                                                          R P
                     F
                        D
                          K
                            Y
                               T
                                 G N
            E E
                 T
                   C
                                                 R C H E
                                 I S
                                          I A N
                        G
                          R
                            G R
                   G
                     Α
                     H E T G G Y M L E C V C L G N G
D H A A G T S Y V V G E T W E K
E G S G R I T C T S R N R C N D
                                               CLGNGKG
40
              R
                 R
                   Ρ
                                          VGETWEKP
                 С
                   F
            Ε
              K
                                                          Q
              С
                 L G
              TWSKKDNRGNLLQCI
SVOTTSSGSGPFTDV
                                                      G N
                                                           G
                                                 CT
          G
            D
            TSVQTTSSGSGPPYGHCVTDSGVV
                                                           Y
                                                 R A
                                                      Α
                                                        V
                                        Ÿ
                                           s
                                                             T
                                             V
                                               G
                                                               Q
                                                    Q
                                                      W
                                                        L
                                                 M
45
          Ρ
        Ρ
                 L
Y
                               С
                                    Ε
                                      T
                                        Α
                                           V
                                             T
                                               Q
                                                 T
                                                    Y
                                                      G
                                                        G
                        G
                          V
                             S
                                 Q
               С
                   G
                      N
          С
            T
                                               Q
F
                                      S N Y
                               S
                                 T
                                             Ε
                                                  D
                                                    QK
                   N
                      D
                        R
                          \mathbf{T}
                             D
                                    T
          Ρ
            F
                                                 LYNNHNY
                               G A
                                        H F
                                             Ρ
                        N S
                            N
                                    L
                                      С
              Т
                 R G
                      G
      V
          V
            Q
                                      QNYDADQKF
                                                           G
                                                                С
            RRDNMKWC
                                    Т
                               GT
          G
                      N E G V M Y R
                                        \mathsf{G} \mathsf{D} \mathsf{Q} \mathsf{W}
                                                  DKQHD
                                      I
50
                   T
          Ε
            I
                 N G R G E W T C I A Y S Q L R D Q C I V D D I T Y F H K R H E E G H M L N C T C F G Q G R G R W K C
            VGNGRGEWTC
```

15

WO 99/31233 PCT/GB98/03766

```
D P V D Q C Q D S E T G T F Y Q I G D S W E K Y V H G V R Y Q C Y C Y G R G I G E W H C Q P L Q T Y P S S S G P V E V F I T E T P S Q P N S H P I Q W N A P Q P S H I S K Y I L R W R P V S I P P R N L G Y
```

or a natural variant thereof using a reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin.

- 43. A method according to any one of Claims 40 to 42, wherein the reagent which can distinguish said polynucleotide from a polynucleotide encoding fibronectin is a polynucleotide according to Claim 31 or 33.
  - 44. A method according to any one of Claims 36 to 43, wherein the cancer is breast cancer.
  - 45. Use of a reagent which can distinguish the polypeptide whose sequence is

```
NLVATCLPVRASLPHRLN
   MLRGPGPGLLLLAVQCLGTAVPSTGASKSK
   RQAQQMVQPQSPVAV
                               KPGCYDN
                                          G
                           S
                            Q S
20
                               YGGSRGF
                                          Ν
   INQQWERTYL
                   G N A
                       L V
                           С
                            T
                              С
                      G N T
                           Y
                              V
                               GDT
                                    YERP
                                          K
                            R
   PEAEET
             C F
               D K Y
                    Τ
               GRGRISC
                            I A N R C H E
                                       G G
                          T
             G A
        T
         С
           Ι
                                          Ε
                   G G Y M L E
                                 LGNGKG
         RRPHET
                            С
                              V
                               С
      T
        M
           CFDHAAGTSYVVGETWEKP
                                         Y
                                          Q
25
        Ε
         K
     Ι
      Α
         CLGEGSGRITCTSRNRCND
                                       Q
                                         D
   V D
        T
        DTWSKKDNRGNLLQC
                                    GNGR
                               Ι
                                 С
                                   T
                            T
                                       Y
                       G
                         Ρ
                           F
                              D
                               V
                                 R A A
                                      V
   ERHTSVQTTSSGS
                              V
                      G
                        V
                         V
                           Y
                               G M
                                    W
                                      L
                                       K T
        PYGHC
                V
                 Т
                   D
                    S
                                   Q
      Ρ
                      Q E T A
                    С
                            V
                              Т
                               Q
                                 T
                                   YGGNSN
30
        T
          С
             G N
                G
                 V
                   S
                               Q D Q K Y S F C F L Y N N H N Y
                       Т
                         SNYE
           Y
                 TDST
          Т
              D R
       Ρ
        F
             N
                 SNGAL
                         CHFP
           RGGN
      V
        Q
          \mathbf{T}
                                         F C
                         QNYDADQKFG
     EGRRDNMKWCGTT
           TINEGVMYRIGDQWDKQHDM
        I
          С
    H E E
      CVGNGRGEWTC
                        ΙA
                           Y
                            S
                              Q
                                L R D
                                    Q
                                      C
                                         V
35
     VNDTFHKRHEEG
                              С
                                Т
                                 С
                                   F
                                       G
                                           G
                        H
                         M L
                            N
                                    G
                                      Q
                                         R
    D P V D Q C Q D S E
                                 S
                                   W
                                    Ε
                                       Y
                                         V
                         Y
                              G
                                D
                                      K
                   T
                     G
                      T
                        F
                           Q
                            Ι
                                T Y P S
                                      S S G P
                                            V E
                   Ε
                     WHC
                         Q
                           Ρ
                            L Q
           G R
              G
                I
                 G
        С
           SQPNSHPIQWNAP
                                QPSHISKYILRW
       Ε
        T
          Ρ
40
          I P P R N L
        S
       V
```

or a natural variant thereof from fibronectin in the manufacture of a reagent for diagnosing cancer.

5

10

15

25

- 46. Use of a reagent as defined in Claim 45, as a diagnostic agent.
- 47. A method of modulating cell migration the method comprising administering an effective amount of a polypeptide according to any one of Claims 10 to 13 to the site where modulation of cell migration is required.
- 48. A method according to Claim 47, wherein the cell is a fibroblast or an endothelial cell.
- 49. A method according to Claim 47 or 48, wherein the site is in a mammalian body.
- 50. A method according to Claim 49, wherein the site is in a human body.
  - 51. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of an agent for modulating cell migration.
- 20 52. Use of a polypeptide according to any one of Claims 10 to 13, for modulating cell migration.
  - 53. A method of healing a wound the method comprising administering to the locality of the wound an effective amount of a polypeptide according to any one of Claims 10 to 13.
    - 54. Use of a polypeptide according to any one of Claims 10 to 13, in the manufacture of a medicament for healing wounds.

3 🍁

5

WO 99/31233 PCT/GB98/03766

55. Use of a polypeptide according to any one of Claims 10 to 13, for healing wounds.

- 56. A pharmaceutical composition comprising a polypeptide according to any one of Claims 10 to 13 and a pharmaceutically acceptable carrier.
- 57. A polypeptide according to any one of Claims 10 to 13 for use in medicine.
- 10 58. A method of preventing scarring comprising administering to the locality of the site where scarring is to be prevented an effective amount of a polypeptide according to any one of Claims 10 to 13.

WO 99/31233

## 1/7

	1	CAAACTTGGT	GGCAACTTGC	CTCCCGGTGC	GGGCGTCTCT	CCCCCACCGT
	51	CTCAA CATGC	TTAGGGGTCC	GGGGCCCGGG	CTGCTGCTGC	TGGCCGTCCA
1	01	GTGCCTGGGG	ACAGCGGTGC	CCTCCACGGG	AGCCTCGAAG	AGCAAGAGGC
1	.51	AGGCTCAGCA	AATGGTTCAG	CCCCAGTCCC	CGGTGGCTGT	CAGTCAAAGC
2	01	AAGCCCGGTT	GTTATGACAA	TGGAAAACAC	TATCAGATAA	ATCAACAGTG
2	.51	GGAGCGGACC	TACCTAGGCA	ATGCGTTGGT	TTGTACTTGT	TATGGAGGAA
3	01	GCCGAGGTTT	TAACTGCGAG	AGTAAACCTG	AAGCTGAAGA	GACTTGCTTT
3	51	GACAAGTACA	CTGGGAACAC	TTACCGAGTG	GGTGACACTT	ATGAGCGTCC
4	01	TAAAGACTCC	ATGATCTGGG	ACTGTACCTG	CATCGGGGCT	GGGCGAGGGA
4	51	GAATAAGCTG	TACCATCGCA	AACCGCTGCC	ATGAAGGGGG	TCAGTCCTAC
5	501	AAGATTGGTG	ACACCTGGAG	GAGACCACAT	GAGACTGGTG	GTTACATGTT
5	551	AGAGTGTGTG	TGTCTTGGTA	ATGGAAAAGG	AGAATGGACC	TGCAAGCCCA
$\epsilon$	501	TAGCTGAGAA	GTGTTTTGAT	CATGCTGCTG	GGACTTCCTA	TGTGGTCGGA
$\epsilon$	551	GAAACGTGGG	AGAAGCCCTA	CCAAGGCTGG	ATGATGGTAG	ATTGTACTTG
7	701	CCTGGGAGAA	GGCAGCGGAC	GCATCACTTG	CACTTCTAGA	AATAGATGCA
7	751	ACGATCAGGA	CACAAGGACA	TCCTATAGAA	TTGGAGACAC	CTGGAGCAAG
8	301	AAGGATAATC	GAGGAAACCT	GCTCCAGTGC	ATCTGCACAG	GCAACGGCCG
8	351	AGGAGAGTGG	AAGTGTGAGA	GGCACACCTC	TGTGCAGACC	ACATCGAGCG
9	901	GATCTGGCCC	CTTCACCGAT	GTTCGTGCAG	CTGTTTACCA	ACCGCAGCCT
9	951	CACCCCCAGC	CTCCTCCCTA	TGGCCACTGT	GTCACAGACA	GTGGTGTGGT
10	001	CTACTCTGTG	GGGATGCAGT	GGCTGAAGAC	ACAAGGAAAT	AAGCAAATGC
10	051	TTTGCACGTG	CCTGGGCAAC	GGAGTCAGCT	GCCAAGAGAC	AGCTGTAACC

# Fig. 1 (part 1)

SUBSTITUTE SHEET (RULE 26)

## 2/7

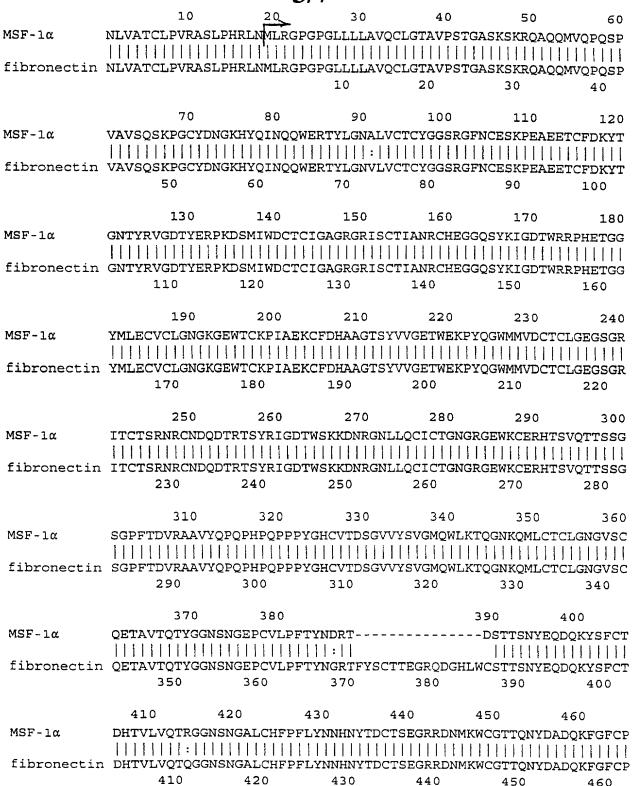
1101	CAGACTTACG	GTGGCAACTC	AAATGGAGAG	CCATGTGTCT	TACCATTCAC
1151	CTACAACGAC	AGGACGGACA	GCACAACTTC	GAATTATGAG	CAGGACCAGA
1201	AATACTCTTT	CTGCACAGAC	CACACTGTTT	TGGTTCAGAC	TCGAGGAGGA
1251	AATTCCAATG	GTGCCTTGTG	CCACTTCCCC	TTCCTATACA	ACAACCACAA
1301	TTACACTGAT	TGCACTTCTG	AGGGCAGAAG	AGACAACATG	AAGTGGTGTG
1351	GGACCACACA	GAACTATGAT	GCCGACCAGA	AGTTTGGGTT	CTGCCCCATG
1401	GCTGCCCACG	AGGAAATCTG	CACAACCAAT	GAAGGGGTCA	TGTACCGCAT
1451	TGGAGATCAG	TGGGATAAGC	AGCATGACAT	GGGTCACATG	ATGAGGTGCA
1501	CGTGTGTTGG	GAATGGTCGT	GGGGAATGGA	CATGCATTGC	CTACTCGCAG
1551	CTTCGAGATC	AGTGCATTGT	TGATGACATC	ACTTACAATG	TGAACGACAC
1601	ATTCCACAAG	CGTCATGAAG	AGGGGCACAT	GCTGAACTGT	ACATGCTTCG
1651	GTCAGGGTCG	GGGCAGGTGG	AAGTGTGATC	CCGTCGACCA	ATGCCAGGAT
1701	TCAGAGACTG	GGACGTTTTA	TCAAATTGGA	GATTCATGGG	AGAAGTATGT
1751	GCATGGTGTC	AGATACCAGT	GCTACTGCTA	TGGCCGTGGC	ATTGGGGAGT
1801	GGCATTGCCA	ACCTTTACAG	ACCTATCCAA	GCTCAAGTGG	TCCTGTCGAA
1851	GTATTTATCA	CTGAGACTCC	GAGTCAGCCC	AACTCCCACC	CCATCCAGTG
1901	GAATGCACCA	CAGCCATCTC	ACATTTCCAA	GTACATTCTC	AGGTGGAGAC
1951	CTGTGAGTAT	CCCACCCAGA	AACCTTGGAT	ACTGAGTCTC	CTAATCTTAT
2001	CAATTCTGAT	GGTTTCTTTT	TTTCCCAGCT	TTTGAGCCAA	CAACTCTGAT
2051	TAACTATTCC	TATAGCATTT	ACTATATTTG	TTTAGTGAAC	AAACAATATG
2101	TGGTCAATTA	AATTGACTTG	TAGACTGAAA	AAAAAAAA	AAAAAA

# Fig. 1 (part 2)

WO 99/31233

PCT/GB98/03766

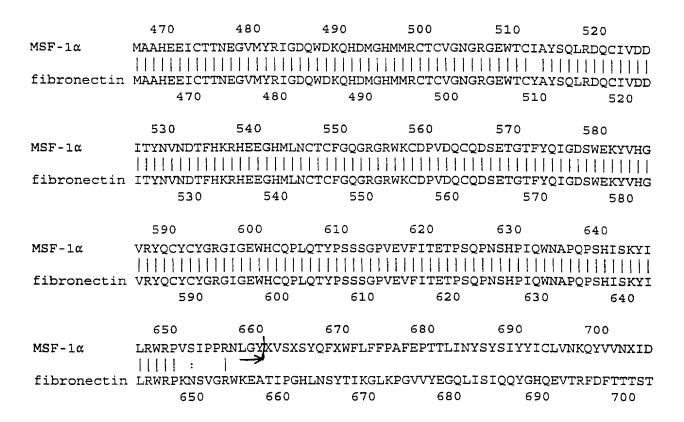
3/7



# Fig. 2 (part 1)

PCT/GB98/03766

#### 4/7



## Fig. 2 (part 2)

	Sequence type:	Binding site:
NLVATCLPVRASIPHRLN	5'untranslated region	
M LRGPGPGLLLLAVQCLGTAVPSTGASKSKR	Signal	
<sup>35</sup> ДАООМ V Q Р Q S Р V A V S Q S K Р G	NH,-terminal segment	
*** Sign byn ^ Crisk hind in Qo (mir r.t. ° l. rin all v ° ring rin r.t. ° rin rin r.t. ° rin r.t.	нынын	Fibrin Heparin S.aureus
ии тѕудттѕя сврето укавуровреруси	Connecting strand	
ээсёртву Сругия с марта марта с с марта марта с	ı	5
11-12 avtrenday Germanner etter gyvlermet krindert B. Mene o nusumiy s bushtd h 11-12 vlev beger germanner etter ette minn hvitdet,segrift den koloninen etteret	# H	Gelatin Celatin
esg ттив ° G ју м KJR I G D Q И D K Q H D ° м G H м и м R KG G G R R R R R R R R R R R R R R R	<b>м м м</b>	
sos gрvвугтетдродия q q голидам в gq голидам в gq голи при к g gr голи при к g	111	
61)V S J P P R N L G Y <sup>642</sup>	Unique sequence	
*VS*SYQF*WFLFFPAFBPTTLINYSYSIYYICLVNKQYVVN*IDL*TEKKKKK	3'untranslated region	

Fig. 3

PCT/GB98/03766

6/7

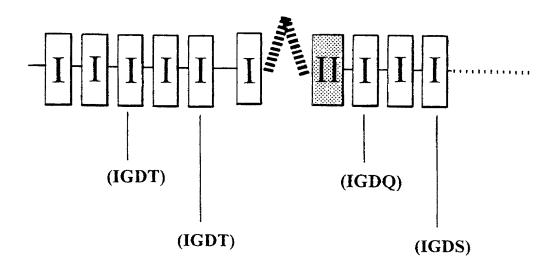
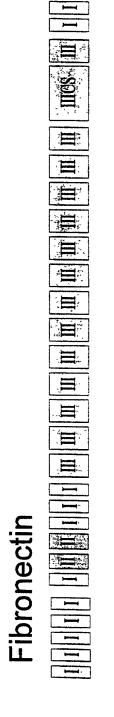


Fig. 4

7/7



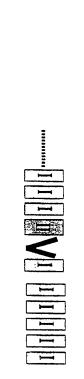


Fig. 5

Please type a plus sign (+) inside this box -	+

PTO/SB/01 (12-97)

Approved for use through 9/30/00 OMB 0851-0032
Patent and Trademark Office, U.S DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains

a valid CMB control Hamber							
	Attorney Docket Num	ber 002.00120					
DECLARATION FOR UTILITY OR DESIGN	First Named Inventor	Schor					
PATENT APPLICATION	COMPL	ETE IF KNOWN					
(37 CFR 1.63)	Application Number	09/581,651					
	Filing Date	June 15, 2000					
☐ Declaration ☐ Declaration  Submitted ☐ OR Submitted after Initial	Group Art Unit	Unknown					
with Initial Filing (surcharge Filing (37 CFR 1.16 (e))	Examiner Name	Unknown					

As a below named inventor, I hereby declare												
My residence, post office address, and citizenship are as stated below next to my name												
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.  POLYPEPTIDES, POLYNUCLEOTIDES AND USES THEREOF												
the specification of which (Title of the Invention)												
is attached hereto OR												
was filed on (MM/DD/	YYYY) 06/15/2000	as Uni	ted States Application	Number or PCT Interna	itional							
Application Number 09/5	581,651 and wa	as amended on (MM/DD/YYY	Υ)	(1)	if applicable)							
I hereby state that I have revie amended by any amendment s	wed and understand the contents	of the above identified spec	ification, including the	claims, as								
	lose information which is material	to natentability as defined in	37 CER 1 56									
Tuditionnedge the daty to see	OSC IIIO MARON WINCOM IS MARON IS	to paternability as defined in										
I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.												
Prior Foreign Application	0	Foreign Filing Date	Priority	Certified Copy Attac	ched?							
Number(s)	Country	(MM/DD/YYYY)	Not Claimed	YES	NO							
9726539.1												
Additional foreign applicatio	n numbers are listed on a suppler	mental priority data sheet PT	O/SB/02B attached he	ereto:								
I hereby claim the benefit unde	er 35 U.S C 119(e) of any United	States provisional application	n(s) listed below.									
Application Number(s)	Filing Date (	MM/DD/YYYY)										
	Application Number(s)  Filing Date (MM/DD/YYYY)  Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.											
	l l											

[Page 1 of 2]

Burden Hour Statement. This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231 DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO

Please type a plus sign (+) inside this box 🛨 📙

PTO/SB/01 (12-97) Approved for use through 9/30/00 OMB 0651-0032



Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

### **DECLARATION**—Utility or Design Patent Application

America, listed I application in the	below and e manner p	under 35 USC 1; , insofar as the subrovided by the first e available between	bject mat paragrap	ter of each of th h of 35 USC 11	e claims of 2, I acknowle	this ap	plication is e duty to d	s not disclose isclose informa	d in the pation which	rior Unit	ed States or PC rial to patentabilit	T International	
U.	S. Pare	nt Application o	or PCT	Parent			ent Filir	_		Parent Patent Number (if applicable)			
PCT/GB98/						(MM/DD/YYYY) 12/15/1998					(п арриосою	<del>/</del>	
Additional t	J.S. or PC	T international appli	cation nu	imbers are listed	on a supplen	nental p	rionity data	sheet PTO/SI	B/02B atta	ched her	eto.		
		by appoint the follow	wing regis	stered practitione	(s) to prosec	ute this	applicatio	n and to transa	act all busi	ness in t			
and Trademark O	ffice conne	ected therewith		Customer Numb	er					→	Place Customer Number Bar Code		
				Registered pract		ne/regis	stration nu	mber listed bel	ow	L	Label here		
Registration Number								Naı	me			stration mber	
Braman, Sus	san J.			34,103			Rogal	lskyj, Peter			38,601		
Weyand, Ka	rla M.			40,223	-								
												<b>)</b>	
Additional re	egistered p	ractitioner(s) named	d on supp	lemental Registe	red Practition	ner Infor	mation sh	eet PTO/SB/02	C attache	d hereto.			
Direct all corres	spondenc	e to.	Custome	er Number or				OR	<b>V</b>	orroeno	ndence addres	s bolow	
			Bar Code	e Label						orrespo	ndence addres	s below	
Name	Susan	J. Braman, Es	q.										
Address	Brama	ın & Rogalskyj	i, LLP										
Address	P.O. E	352 Sox 352											
City	Canan	daigua				State NY ZIP 1442			24-0352				
Country	USA			Telephone	716-3	393-3	002		Fax	716-	393-3001		
further that these	statement	atements made hen is were made with the willful false statemen	ne knowle	edge that willful fa	alse statemer	its and	the like so	made are pun	ishable by				
Name of Sole	e or Firs	t Inventor:					A petition	ı has been fil	ed for thi	s unsigr	ned inventor		
Gi	ven Nam	e (first and middle	e [if any]	)		Family Name or Surname							
Seth Lawrer	nce					Sc	hor						
Inventor's Signature	dr	-				Date	14.03-00						
Residence City Dundee				State	C	Country United Kingdom			m	Citizenship	GB		
Post Office Address Unit of Cell and Molecular Biology, The Dental School													
Post Office Ad	Idress	University of	Dunde	ee									
City		Dundee	State		DD1 4HR			Cou	Country UK				
Additional in	nventors	are being named	on the	1 su	polemental	Addıtır	onal Inve	ntor(s) sheet	(s) PTO/	SB/02A	attached heret	0	



Please type a plus sign (+) inside this box ---

PTO/SB/02A (3-97)
his box 

+ Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office, U.S DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number

#### **DECLARATION**

#### ADDITIONAL INVENTOR(S) Supplemental Sheet Page 1 of 1

Name of Addition	al Joint Inventor, if any		A petition has been filed for this unsigned inventor							
Given Na	me (first and middle [if any])			Family Name or Surname						
Ana Maria	S	Schor								
Inventor's Signature	Aan Shal							Date	,	14.08.00
Residence City	Dundee	State		С	ountry	United Kingd	om	Citizens	hip	ES
Post Office Address	Unit of Cell and Molecula	r Biolo	gy, The De	ntal	Schoo	, GB,	X. ——			
Post Office Address	dress University of Dundee									
City	Dundee	State			ZIP ]	DD1 4HR	Country	, UK		
Name of Addition	al Joint Inventor, if any:		[	A	petitic	on has been file	d for th	is unsigi	ned in	ventor
Given Nar	me (first and middle [if any])					Family Nar	ne or S	Surname		
Inventor's Signature			<b>41.4.1</b>					Da	Date	
Residence: City	300 000 000 000 000 000	State		Country Citizenship						
Post Office Address										
Post Office Address		1								
City		State			ZIP		Coun	itry		
Name of Addition	al Joint Inventor, if any:		[	A	petitio	n has been filed	s been filed for this unsigned inventor			
Given Nar	me (first and middle [if any])			Family Name or Surname						
Inventor's Signature								Da	ite	
Residence <sup>-</sup> City	dence <sup>.</sup> City State Country							Crtizenship		
Post Office Address										
Post Office Address										
City State ZIP Country										

Burden Hour Statement This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231 DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO Assistant Commissioner for Patents, Washington, DC 20231

